ENERGY NUTRITION AND BIOLOGICAL ASPECTS OF ENERGETICS IN SELECTED CULTIVABLE FISH SPECIES

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

ZOOLOGY

BY

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ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1993

ABSTRACT

The thesis incorporates results of the studies conducted on some aspects of energy nutrition in selected cultivable finfish species. It also presents data on seasonal changes in biological indices and calorific value of somatic and gonadal tissues of a catfish, and attempts to establish relationships between these parameters.

Protein and energy maintenance, and optimum feed requirements were assessed for the catfish, Clarias batrachus. Linear ($r = 0.985$) increase was observed in daily average growth increment up to ration level of 4% ($Bw.day^{-1}$), corresponding to 6.03 g protein/($kg^{0.8}Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}$ $Bw.day^{-1}$). Maintenance requirements, obtained by regressing daily average growth increment to zero, were 0.942/($kg^{0.8}$ $Bw.day^{-1}$) for protein and 8.605 kcal/($kg^{0.8}Bw.day^{-1}$) for energy. Net gains in muscle protein and energy also depicted linear increase ($r = 0.975$) with feeding levels up to 6.03 g protein/($kg^{0.8}$. $Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}Bw.day^{-1}$). On fitting the above data to regression equation, the amount of dietary protein and energy required to maintain a constant amount of body protein and energy, values of 1.005 g/($kg^{0.8}Bw.day^{-1}$) and 10.06 kcal/($kg^{0.8}Bw.day^{-1}$) were obtained for protein and energy, respectively. The optimum feeding rate for this species, as evident from specific growth rate and conversion efficiencies, appeared to be 3% ($Bw.day^{-1}$) at 30±2°C. Moisture and lipid contents of muscle were found significantly ($P<0.05$) affected by ration levels.

Apparent energy digestibility coefficient of nine different feedstuffs were determined for two size-classes of Indian major carps, Labeo rohita and Cirrhinus mrigala, using chromic oxide as indigestible marker. Among the ingredients tested, rice polish showed the lowest and soybean oilcake the highest digestibility in both the species. The influence of fish size on energy digestibility was found insignificant ($P < 0.01$). Categorywise, irrespective of fish size, plant and animal origin feedstuffs showed insignificant ($P < 0.01$) difference

in their digestibility. With the exception of animal origin feedstuffs in larger size class, differences in digestibility of feedstuff, irrespective of source, were insignificant ($P < 0.01$) between the two species for both the size groups.

Influence of feeding varying levels of dietary energy has been examined on growth, utilization efficiency and carcass composition of C. mrigala. Weight gain was found significantly ($P < 0.01$) affected by dietary energy levels, following a quadratic pattern. A second degree polynomial curve indicates that an energy level of 355.00 kcal/100 g, corresponding to an E/P ratio of 8.87 (kcal g^{-1}), would produce the maximum weight gain. Efficiency of feed and protein conversion, in terms of FCR, PER, and PPV, increased only up to a certain level of energy incorporation. Carcass composition was significantly ($P < 0.01$) affected by dietary energy. The relationship between energy levels and various carcass components has been pointed out.

Two levels of dietary protein (30 and 40% C.P.), with three energy levels each, were used to determine the optimum energy to protein ratio in fingerling C. mrigala. Factorial analysis of variance indicated that although weight gain, feed and protein utilization efficiencies were significantly affected by dietary energy and protein levels, the energy and protein conversion efficiencies in this species were influenced both by the levels of energy and protein in diet, and their interaction. Best growth and feed conversion were obtained with an average of 360.77 kcal/100 g at 40% C.P. diet (E/P, 8.93) while maximum protein utilization and conversion occurred with similar energy but low (30%) protein in diet (E/P, 12.13). Although significant influence of dietary energy was evident on all the body components of fish, the influence of protein was insignificant.

Effect of dietary energy and protein, and their interaction on growth, utilization efficiencies and body composition was evaluated in young C. batrachus fed three levels of protein (25, 35 and 40% C.P.), each at two metabolizable energy levels (360.3±5.72 and

407.1±4.4 kcal/100 g). Growth of fish, in terms of live weight gain (%), increased with increasing dietary protein ($r = 0.95$; $P < 0.05$) at both the energy levels. A general reduction in growth rate occurred with increase in energy density of diets at each protein level. Within isocaloric diets, SGR (%) exhibited a linear negative correlation ($r = -0.96$; $P < 0.10$ for 360 kcal and $r = -0.99$; $P < 0.005$ for 407 kcal/100 g) with E/P ratio. Total mean feed consumed was found influenced by dietary energy and protein levels and their interaction. FCR, PER and ECE were affected only by dietary energy and protein levels while PPV was influenced both by the levels of energy and protein as well as their interaction. When isocaloric diets were compared, PER and PPV exhibited a direct, whereas FCR and ECE indicated an inverse correlation with E/P ratios. Carcass composition was influenced by dietary energy but influence of dietary protein or energy-protein interaction was not evident.

Changes in annual calorific value of somatic and gonadal tissues, and biological indices of mature catfish, Heteropneustes fossilis have been investigated. The biological indices studied showed significant annual changes in the two sexes, except condition factor (K) in male. Excepting the gonado-somatic index, the various indices showed insignificant differences between the sexes. Variations in calorific value of trunk muscle, liver, gut and gonad were found significant over the year. The pattern of changes in the calorific value of muscle, gut and liver were also more or less identical in the two sexes. Calorie deposition in testes remain significantly lower than in the ovaries.

The significance of various observations, and usefulness of data have been discussed.

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CHECKED-2002

TO

MY

PARENTS

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Professor

I certify that the work entitled, "**Energy Nutrition and Biological Aspects of Energetics in Selected Cultivable Fish Species**" is completed under my direct supervision by **Mr. Md. Abul Hassan.** The work is original and independently pursued by the candidate. It embodies some interesting observations contributing to the existing knowledge on the subject.

I permit the candidate to submit the work for the award of the degree of **Doctor of Philosophy in Zoology** of the Aligarh Muslim University, Aligarh.

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ACKNOWLEDGEMENTS

I wish to express my deepest sense of gratitude to **Professor A.K. Jafri** for his brilliant supervision, valuable suggestions, constructive criticisms, and carefully going through the work.

I am indebted to **Professor John E. Halver**, University of Washington, U.S.A., for expert suggestions of his marvellous repertoire of knowledge; and to **Professor E.A. Huisman**, Department of Fish culture and Fisheries, Wageningen, the Netherlands, for his perusal and valuable critical assessment of some portion of the work.

Thanks are also due to **Professor M.M. Agarwal**, Chairman, Department of Zoology, Aligarh Muslim University, Aligarh, for providing the necessary laboratory facilities.

I am far too grateful to my laboratory colleagues, **Dr.(Ms.) Shabana Firdaus, Dr. Mukhtar A. Khan, Mr. Farooque Anwar** and **Mr. Erfanullah** for their unfailing cooperation. Special thanks are due to **Ms. Afreen Alvi, Ms. Rana Samad** and **Ms. Nazura Usmani** for their unflinching support at crucial moment when it needed to hustle on to finish the task with exactness.

Warmest thanks are due to all my colleagues & friends, especially **Dr. Sudhir Singh, Mr. Jamal Ahmad, Mr. Khawaja Jamal, Dr. Badrudduza, Mr. Niamat Ali, Mr. Gulam M. Khan** and **Mr. Azharul Islam** for their continued inspiration and generosity.

It is my humble duty to thank my parents, without whose blessings and forbearance it was difficult to continue the endeavour with perseverance.

Last but not the least, thanks are also due to **Mr. Kafil A. Khan** and **Mr. Zia-ur-Rehman Khan Sherwani** for neat and careful typing of the manuscript.

Financial assistance from **Far Eastern Regional Research Office,** U.S. Department of Agriculture and Indian Council of Agricultural Research, New Delhi, is gratefully acknowledged.

MD. ABUL HASSAN

CONTENTS

GENERAL INTRODUCTION

Fish nutrition, a relatively young discipline, in terms of accumulation of knowledge and level of sophistication, has advanced fairly rapidly after the pioneering work of Halver (1972) on amino acid and vitamin requirement of salmonid fishes (Halver, 1957; NRC, 1973), with information on the nutrition of almost all the aquaculturally important temperate fishes reaching close to precision. Based on nutritional requirements, diets have also been developed for rearing the various growth stages of these fishes. Among tropical fishes, channel catfish and common carp have been extensively studied for their nutritional requirement. The information generated on the subject over the years have been extensively reviewed (Halver, 1972, 1979[a,b], 1985, 1989; Cowey and Sargent, 1972, 1979; Ketola, 1977, 1980; Cowey, 1979; Castell, 1979; Lall, 1979, 1989; Watanabe, 1982; Millikin, 1982; Jauncey, 1982[b]; Tacon and Cowey, 1985; Wilson, 1985, 1989; Wilson and Halver, 1986; Lovell, 1977, 1989; Sargent et al., 1989; Smith, 1989; Tacon, 1990 and NRC, 1973, 1977, 1981, 1983).

Although historically aquaculture existed in Asia from times immemorial, as a science and industry, it is still considered to be in its infancy (Pillay, 1986). India is bestowed with vast and varied resources, in the form of water spread, candidate species, cheap and plenty raw materials, and manpower for aquaculture development, it has not yet been able to tap its

potential to the fullest. With the current level (4.2 m tonnes) of its fish production, the per capita availability of fish in the country is still around 3.2 kg/annum against the stipulated minimum of 10 kg. It is projected that, to provide a stipulated minimum requirement, India would require around 13 million tonnes of fish, with 7 million tonnes originating from aquaculture. The unpredictable pattern of capture fisheries and increasingly greater demand for export oriented fishery product necessitate the need for the intensification (aquaplosion) of aquaculture practices. Finfish aquaculture in India predominantly involves the culture of three species of major carps and is practised mainly through semi-intensive mode of management with varying levels of supplementary feeding. A major constraint in the development of intensive aquaculture of these fishes is the absence of practical feed precisely tuned with the nutritional needs of the concerned species. Since feed constitutes almost 60-70% of operational cost in such practices, nutritional research becomes increasingly important for cost effective diet development. Precise knowledge of nutrient requirement thus becomes a prerequisite for formulating a nutritionally adequate practical diet. Basic nutritional requirements of most of the cultivable Indian fish species, including Indian major carps, are not fully understood. Realising the gravity of the situation, a recently concluded US-India sponsored International Symposium on Aquaculture Research Needs for 2000 AD, recommended (Wang and Dehadrai, 1992) the need for intensive research on nutrient requirement of these fishes leading ultimately

to the development of supplementary/complete feed for their culture.

As in any industry, in aquaculture as well, the level of profitability depends, to a great extent, on the optimization of various operations. Since feed, irrespective of whether a culture is intensive or semi-intensive, is a major, if not the highest, operational cost in most practices, a number of approaches, either singly or collectively, are adopted to increase profitability. To minimise feed cost, and check water quality deterioration, due to excessive feeding, knowledge of optimum feeding rate is essential for rearing a particular fish species. The influence of feeding level on growth, feed efficiency and chemical composition of fish has been investigated in various species (Paloheimo and Dickie, 1966; Brett et al., 1969; Pandian and Raghuraman, 1972; Huisman, 1976; Staples and Nomura, 1976; Cho et al., 1976; Wurtsbaugh and Davis, 1977[a,b]; Huisman et al., 1979; Reddy and Katre, 1979; Reinitz, 1983[a,b]; Hogendoorn, 1983; Hogendoorn et al., 1983; Goolish and Adelman, 1984; Machiels and Henken, 1986; Storebakken and Austreng, 1987[a,b]; Hung and Lutes, 1987). Information on optimum feeding rate is equally important for nutrient requirement determination of the concerned species as requirement for major nutrients are known to be affected by the rate of feeding (Tacon and Cowey, 1985; and Talbot, 1985). Available information also indicate that ration level influences nutrient digestibility (Vens-Cappel, 1978; Windell et al., 1978; Henken et al., 1985 and Storebakken and Austreng, 1987[b]). The optimum ration level in fish

may vary with size and environmental conditions (Huisman, 1976; Wurtsbaugh and Davis, 1977[a,b] and Hung and Lutes, 1987). A review on the above aspects is given by Brett (1979).

Maintenance energy/nutrient requirement is the amount required to maintain a state of equilibrium, in terms of energy/ nutrient, in the body tissue. The less the energy or nutrient expended towards maintenance, the more efficient will be their utilization for growth. In fish, maintenance energy requirements are lower than those of terrestrial animals, since fish do not have to regulate their body temperature, and expand less energy to maintain their position in water (NRC, 1983). This qualifies fish as efficient converters of nutrient to flesh. Several fish species have been studied for their energy and protein maintenance requirements (Huisman, 1976; Cho et al., 1976; Wurtsbaugh and Davis, 1977[a,b]; Kaushik et al., 1981; Kaushik and Luquet, 1984; Gatlin et al., 1986; Storebakken and Austreng, 1987[b]; Brown et al., 1990; and Storebakken et al., 1991). Being poikilothermic, maintenance requirement in fish is reported to be markedly influenced by changes in environmental temperature (Winberg, 1956; Brett et al., 1969; Kelso, 1972; and Niimi and Beamish, 1974).

Studies on nutrient digestibility in fish are relatively recent and commenced as sequel to intensification in aquaculture which go hand in hand with development and dependence on artificial diets (De Silva, 1989). A feedstuff which may appear from its chemical composition to be an excellent source of nutrient

may be of little actual value if it is not digested and absorbed by the target species. Knowledge of nutrient availability from various feedstuffs is desirable for diet development so that effective substitution of one ingredient by the other may be achieved when required. Digestibility studies are thus pivotal to feed development in aquaculture. Information on the digestibility of feed ingredients or basic dietary nutrients are also essential for fish energetic studies. Evaluation of feedstuffs for their nutrient/energy digestibility have been made in several fishes (Andrews et al., 1978; Cho and Slinger, 1979; Pfeffer, 1982; Ellis and Smith, 1984; De Silva and Perera, 1984; Schmitz et al., 1984; Law, 1984, 1986; Hajra, 1985; Wilson and Poe, 1985; Law et al., 1985; Kirchgessner et al., 1986; Ferraris et al., 1986; Hanley, 1987; De Silva et al., 1988; Hossain and Jauncey, 1989; Querijero and Chiu, 1989; Kamaruddin et al., 1989; and Stone et al., 1989). Many investigators have looked into the factors affecting nutrient digestibility in fish, such as age, size, density of fish, quantity of food, frequency of feeding, previous level of nutrition, forced versus ad libitum feeding, water temperature, salinity, ingredient processing and presence of toxins etc.(Pandian,1967;Shrable et al.,1969; Hastings, 1969; Cho and Slinger, 1979; Vens-Cappel, 1978; Windell et al., 1978; Andrews, 1979; Brett and Groves, 1979; Atack et al., 1979; Pandey and Singh, 1980; De Silva and Perera, 1984; Steffens, 1985; Henken et al., 1985; and De Silva, 1989).

Knowledge of energy requirements in fish remained slow to accumulate. Fish require much less energy for protein synthesis than terrestrial farm animal (Lovell, 1989). Being aquatic and poikilothermic, fish have several other advantages with respect to their energy requirement.

As common with other animals, with the exception of human beings, fish have a sate point as far as food consumption is concerned (NRC, 1983). They stop feeding when their energy requirements are met with. Fed on diet with a satisfactory nutrient balance, fish can compensate for a low dietary energy density by eating more of it, up to the limits of the physical capacity of their digestive tract. Conversely, on a high energy density diet, fish would require less feed to satisfy their energy needs before meeting with the requirements for other vital nutrients. Summarily, feed consumption and dietary energy density in fish appear inversely related to each other.

Provision for an optimal balance of energy nutrients in diet is important because both excess or deficiency of nonprotein energy sources (lipid and carbohydrate) may lead to reduced growth rate. In the absence of sufficient nonprotein energy sources in the diet, fish may catabolize protein (amino acids) for various energy expenditures (basal metabolism and voluntary activity) resulting in low growth rate. Similarly, feeding high energy density diet may restrict feed consumption, thereby leading to insufficient quantity of protein ingestion which may fail to satisfy demands

for maximal rate of protein synthesis and growth.

Absolute figure on optimum energy requirements are difficult to define in fish nutrition because dietary energy as such is not directly utilized for growth, in terms of protein accretion, it rather helps in the utilization of dietary protein (amino acid) to be laid down for growth. Consequently, dietary energy requirements in fish are often expressed as a function of dietary protein. Many studies were, therefore, directed towards understanding the interaction between dietary protein and energy (Ringrose, 1971; Page and Andrews, 1973; Lee and Putnam, 1973; Garling and Wilson, 1976; Takeuchi et al., 1979; Bromley, 1980; Zeitler et al., 1984; Machiels and Henken, 1985; Daniels and Robinson, 1986; Henken et al., 1986; Barrows et al. 1988; Reis, et al., 1989; Parazo, 1990; Shiau and Huang, 1990; Jobling et al., 1991; and El-Sayed and Teshima, 1992). Several investigators have also looked into interactions between dietary lipid or carbohydrate (as nonprotein energy sources) and protein (Garling and Wilson, 1977; Murray et al., 1977; Reinitz et al., 1978; Millikin, 1983; Tabachek, 1986; Ellis and Reigh, 1991; De Silva et al., 1991; Serrano et al., 1992).

Knowledge of dietary energy density is also considered important in view of its influence on body composition. Excessive energy intake can lead to deposition of large amounts of body fat (Page and Andrews, 1973; Zeitler et al., 1984).

Several factors, including species, water temperature,

size, age, physiological activity, composition of diet, light exposure and environmental stress, are known to influence the energy requirement of fish.

Since success in breeding depends, to an extent, on greater investment towards gonadal development, the energy requirements of fish increase during periods of gametogenesis and spawning. In female, maturation of oocytes, an aspect of constructive metabolism, requires appreciable energy expenditure. It is opined that in male, although gonadal anabolism is limited, catabolic functions such as nest building, courtship and care of eggs, possibly equate the high anabolic expenditure of female fish (Miller, 1984; and Rogers, 1986). Energy drain due to reproduction may be so great that adults may often perish after spawning or their nutritive quality get depleted. In aquaculture, therefore, strategy should be to harvest the produce before much energy is diverted from growth to gamete production, thereby reducing the feed conversion efficiency.

Prior to spawning, the mass of genital products may constitute a greater (approximately upto 30%) portion of the total body weight of a fish. Production of gametes and spawning are energetically much expensive, and if energy intake is insufficient to fully meet the cost of gonadal growth and somatic maintenance the balance is met with the stored body energy (Love, 1960, 1970; Nikolskii, 1969; Shulman, 1974; Eliassen and Vahl, 1982; Adams et al. 1982; Henderson et al., 1984; Chellappa et al., 1989). The

phenomenon is reflected in noticeable seasonal changes in tissue (muscle and liver) energy content. The importance of energy budget, which typically compartmentalizes energy allotted for growth, metabolism and reproduction, assumes significance in developing strategies for fishery management practices. To establish relationship between changes in various biological indices and diversion of energy for gonadal development, and to understand the role played by energy depots, information pertaining to annual changes in calorific value as well as physical measurements of somatic and gonadal tissue seem essential.

Although a good account of the work on energetics of reproduction is available (Wootton, 1985), it mostly relates to information on fishes from the temperate region. Season-related changes in chemical composition/energy nutrients of fish have been exhaustively reviewed (Love, 1970, 1980). With few exceptions (Jafri, 1968[a,b], 1969; Jafri and Khawaja, 1968; Shreni, 1980), literature on this subject is also dominated by temperate fishes (MacKinnon, 1972; Newsome and Leduc, 1975; Hopkirk et al., 1975; Craig, 1977; Foltz and Norden, 1977; Medford and Mackay, 1978; Pierce et al., 1980; Eliassen and Vahl, 1982; Adams et al., 1982; Flath and Diana, 1985; Rogers, 1988; Chellappa et al., 1989; Dygert, 1990; and Smith et al., 1990).

As already stated, nutritional studies on Indian fishes are relatively few. The first systematic study on nutritional requirements of Indian major carps, using purified test diets

fortified with vitamins, and minerals, was undertaken by Sen et al. (1978). Work cited in literature are mostly on protein requirement of carps and catfishes (Singh et al., 1987; Ray and Patra, 1987; Swamy et al., 1988; Singh and Bhanot, 1988; Akand et al., 1989; Khan and Jafri, 1990, 1991; and only a few workers have investigated the lipid and carbohydrate requirements of these fishes (Singh and Bhanot, 1988; Swamy, 1988; Anwar and Jafri, 1994; Erfanullah and Jafri, 1993). The only information on amino acid requirement is that of Ravi and Devaraj (1991) on Catla catla. Studies on ingredient digestibility in Indian major carp is restricted to C. mrigala (Singh, 1992). Information on energy and protein maintenance requirement, optimum ration level, coefficient of energy digestibility, and energy/protein interaction seem almost lacking in Indian cultivable fishes. Similar paucity of information exists on biological aspects of energetics of these fishes. This study was, therefore, undertaken with a view to generate information on the above subject, and the findings are presented in the form of this thesis.

The thesis consists of two parts :

Part I contains information on energy and protein maintenance requirements, optimum ration level, coefficients of energy digestibility; influence of dietary energy density and energy/protein interaction on growth, utilization efficiency and carcass composition of some selected cultivable finfish species.

Part II embodies information on biological aspects of energetics of a catfish.

The study, in addition to contributing to the existing knowledge on the subject, provides data which would be of interest to fish nutritionists, feed technologists, farmers, fishery managers and biologists engaged in research and culture of the species investigated.

GENERAL METHODOLOGY

I. Source of fish stock/acclimation

Induced bred fry and fingerling of the Indian major carps, Labeo rohita (Hamilton) and Cirrhinus mrigala (Hamilton), were obtained from Uttar Pradesh Fish Seed Cooperative Production Centre, Kolahar, Mathura. These were transported to the research station in oxygen filled polythene bags, given a prophylactic dip in $KMnO_4$ solution (1:3000) and stocked in earthen outdoor fish ponds (12x6x1.5 m). During this period the fishes were fed to satiation a mixture of soybean and mustard oil cake, wheat bran and fish meal (35% C.P.), twice daily, at 0800 and 1600 h. Desired number of fishes were transferred to wet laboratory and acclimated to casein-gelatin (H-440) semipurified or feedstuff based diets for 2-week in 70 l flow-through type (1 l/m) high density polyvinyl circular troughs.

Young Clarias batrachus (Linnaeus), procured from a local fish farm of Aligarh (Lat. 27°34'30" N, Long. 78°4'26" E), were given a dip in $KMnO_4$ solution (1:3000) and stocked in outdoor cement cistern (1x1x1 m) fitted with flow-through system (water exchange rate and volume being 1 l/m and 700 l, respectively) and supplied with ground water. During acclimation, the fish were fed in the manner described above.

Samples of Heteropneustes fossilis were obtained from local ponds during the first week of each month, over a period of one year (March, 1988 to February, 1989), for study on biological aspects of energetics.

II. Preparation of experimental diets

Casein-gelatin based semipurified test diets were formulated for various experiments. Calculated quantities of dietary ingredients were weighed on a sensitive electronic balance (Precisa-120 A). A known quantity of water was then taken in a stainless steel attachment of Hobart electric mixer and heated to 80°C. Gelatin was dissolved into it with slow stirring and heating the content on to a double boiler unit. The mixer bowl was removed from heating, dextrin and mineral mix added to it, and the content blended in Hobart mixer while still in lukewarm state. This was followed by the addition of remaining dietary ingredients like vitamin and oil mix (2:1, corn and cod liver oil), excepting carboxymethyl cellulose which was added in the last. The final diet, about the consistency of bread dough, was poured into a teflon-coated pan and placed into a refrigerator to jell. The prepared diet was in the form of moist (50 to 52% moisture) cake from which small cubes were cut and stored (-20°C) in sealed polythene packs until used.

The mineral and vitamin premixes used (Table 1-2) were the same as given by Halver (1989).

III. General experimental design/feeding trial

Fish of the desired size and number, sorted out from the acclimated lots, were stocked in triplicate groups in 70 l polyvinyl

circular troughs (water volume 55 l) provided with continuous flow of ground water. The water exchange rate in each trough was maintained at 1-1.5 l/min. Each morning, before the commencement of feeding, fecal matter was siphoned off from the experimental troughs. The feeding schedule and level were chosen after carefully observing the dietary intake and feeding behaviour of fish. A 12:12 light and dark cycle was maintained with incandescent lighting.

Fish were fed the experimental diets, six days a week, in the form of moist cake, and the ration level calculated as dry feed to wet fish weight after estimating the moisture content of diet. Mass weight (g) of fish was recorded weekly and the amount of ration readjusted accordingly. For length and weight measurements, fish were anaesthesized with Ayerst's Finquel (tricaine methanesulphonate; 1:10,000). No feed was offered to the fish on the day of weekly measurement when the troughs were also scrubbed thoroughly and rinsed with $KMnO_4$ solution. A record of the dissolved oxygen and water temperature, based on daily measurements, was separately maintained.

IV. Proximate analyses

Proximate composition of ingredients and body/carcass was made using standard techniques (AOAC, 1984).

(i) Estimation of moisture

A weighed quantity of finely ground/homogenized sample was taken in a pre-weighed *silica crucible* and placed in an oven (100°C)

for 24 h. The crucible containing the dried sample was directly transferred to a desiccator, allowed to cool and reweighed. The entire process was repeated till two successive weighings produced least difference in weight. The loss in weight was expressed as percentage of moisture.

(ii) **Estimation of ash**

A known quantity of finely powdered sample was taken in a pre-weighed silica crucible and incinerated in a muffle furnace (600°C) for 2-3 h till the sample became carbon-free. The crucible containing the incinerated sample was transferred to a desiccator, allowed to cool and reweighed. The loss in weight was expressed as percentage of ash.

(iii) **Estimation of crude fat**

A continuous soxhlet extraction technique, using petroleum ether (40-60°C B.P.) as solvent, was employed. Weighed quantity of finely ground sample was taken in Whatman fat extraction thimble, plugged with cotton and introduced into the soxhlet. A clean, dry soxhlet receiver flask was weighed and fitted to the soxhlet assembly for extraction. The extraction was continued for 10-12 h. After extraction, the flask was removed and kept in hot air oven (100°C) to evaporate the traces of solvent. It was then transferred to a desiccator, cooled and reweighed. From the increase in the weight of the flask, the quantity of fat extracted was calculated and expressed in percentage.

(iv) **Estimation of crude protein**

The technique employed for estimating the crude protein content was based on a slight modification of Wong's micro-Kjeldahl method, as adopted by Jafri, 1965. The principle involved digesting the sample with N-free sulphuric acid in the presence of potassium persulphate, used as a catalyst, which converts the nitrogenous compounds to ammonium sulphate. This was then nesslerized and the colour developed, due to the formation of a complex compound (NHg_2I), was measured spectrophotometrically. The optical density obtained was read off against a standard curve of NH_4SO_4 for nitrogen estimation. To calculate the total crude protein in the sample, the amount of nitrogen was multiplied with the conventional protein factor (6.25).

A known amount (0.1-0.5 g) of dry, powdered sample was taken in a Kjeldahl flask containing 5 ml of sulphuric acid (1:1) and heated till fumes appeared. After cooling, 0.5 ml saturated potassium persulphate was added to digesting mixture, and the heating continued till the solution became water clear. After cooling, the digested mixture was transferred to a 50 ml volumetric flask and made to volume with double distilled water. From this, 0.5 ml of the aliquot was taken in a test tube and 0.1 ml of sulphuric acid (1:1) and saturated potassium persulphate added to it. The volume was raised to 3 ml with distilled water. The solution was then nesslerized, using 7 ml of Bock and Benedict's Nessler reagent (Oser, 1965), mixed well and allowed to stand for 10 min before measuring

the absorbance. The absorbance was measured after setting the instrument to zero absorbance with a blank, at 480 m μ. The blank was prepared, without the sample aliquot, in the manner described above. The amount of nitrogen was obtained by reading the optical density against a standard calibration curve (Fig.1), prepared by plotting absorbance values against graded concentration of ammonium sulphate. The nitrogen value was multiplied with 6.25 to obtain the amount of crude protein.

(v) **Estimation of crude fibre**

Crude fibre was measured as loss on ignition of dried residue, remaining after digestion of sample with standard solution of sulphuric acid and sodium hydroxide, under carefully controlled condition.

A weighed quantity of dry, powdered and fat-free sample was taken in a spoutless conical flask fitted to a reflux condenser. Boiling distilled water was added to it, followed by addition of 25 ml sulphuric acid (1.25% w/v) and the content mixed well. The volume of the mixture was raised to 200 ml and the content boiled for 30 min. Broken Alundum crucible chips were added to prevent bumping. The solution was removed from heating and the residue collected over a sintered disc filter and washed at least three times with boiling water. The residue was again transferred to the beaker and to this was added sodium hydroxide (1.25% w/v), and the volume raised to 200 ml with boiling water. Boiling was continued for

another 30 min. At the end of boiling, the mixture was filtered and the residue washed with boiling sulphuric acid, followed by three washings with boiling water. To remove traces of water, the residue was washed with methanol, transferred to silica crucible and dried (100°C) to a constant weight. It was then allowed to cool in a desiccator, weighed and ignited for 2-3 h in a muffle furnace (600°C). The crucible containing the incinerated sample was transferred to a desiccator for cooling and reweighed. The loss in weight of the residue after ignition was expressed in percentage as the amount of fibre, on dry weight basis.

V. Estimation of gross energy

Gross energy was determined by direct calorimetry. A known quantity of finely ground, dried sample was taken into a metallic crucible and compressed to a pellet. Combustion was carried out on ballistic (adiabatic) bomb calorimeter (Gallenkamp and Loughborough Co. Ltd., Loughborough, England). Before carrying out the energy estimation, the calorimeter was calibrated by establishing the relationship between the galvanometer deflection and the amount of heat released on combustion of a measured quantity of thermochemical grade benzoic acid (6.32 kcal g^{-1}). The calorific value was expressed as kcal g^{-1}.

VI. Estimation of chromic oxide

Chromic oxide was estimated using acid digestion technique (Frukawa and Tsukahara, 1966). Diet and fecal matter samples,

containing the chromic oxide as a marker, were taken in a Kjeldahl flask and digested by heating in 5 ml conc. nitric acid for about 30 min to remove organic matter. In order to prevent the content from complete drying, more nitric acid was added, if required. The mixture was removed from heating, allowed to cool, and 3 ml of perchloric acid (60%) added to it. Heating was resumed till no more fumes evolved, indicating disappearance of all traces of nitric acid. Oxidation of chromium oxide to dichromate was indicated by the development of a brilliant golden yellow colour. After cooling, the content was washed thoroughly into a 100 ml volumetric flask and the volume made to mark with distilled water. After keeping the solution for 10 min at room temperature, for complete colour development, the optical density was measured at 350 mμ on a spectrophotometer. The concentration of chromic oxide in the sample was measured as absorbance.

All spectrophotometric measurements were made on microprocessor-controlled split beam spectronic 1001 spectrophotometer (Milton Roy Company, USA).

VII. **Assesment of growth and conversion efficiencies**

Calculation of various growth parameters, and nutrient conversion efficiencies were made according to the following standard definitions (Millikin, 1983; Tabachek, 1986; and Parazo, 1990):

$$\text{Percent increase in live weight (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\log_e W_2 - \log_e W_1}{D} \times 100$$

where,

W_1 = Initial mass weight (g)

W_2 = Final mass weight (g)

D = Duration of the feeding trial (days)

$$\text{Feed conversion ratio} = \frac{\text{Total feed intake (g)}}{\text{Live weight gain (g)}}$$

$$\text{Gross growth efficiency (\%)} = \frac{\text{Live weight gain (g)}}{\text{Total feed intake (g)}} \times 100$$

$$\text{Protein efficiency ratio} = \frac{\text{Live weight gain (g)}}{\text{Total protein intake (g)}}$$

Protein productive value (%) = 100 X (Final wet weight X Final percent body protein) - (Initial wet weight X Initial percent body protein)/(Amount of diet fed/No. of fish per trough) X % crude protein in diet.

Energy conversion efficiency (%) = 100 X [(Final wet weight X Final body crude energy (kcal g^{-1})] - [(Initial wet weight X Initial body crude energy (kcal g^{-1})]/[(Amount of diet fed/No. of fish per tank) X Total energy in diet (kcal g^{-1})].

Specific rate of change in protein (% day^{-1}) = 100 X (In Final percent body protein - In Initial percent body protein)/ No. of days

Specific rate of change in energy (% day^{-1}) = 100 X [(In Final body crude energy (kcal g^{-1}) - In Initial body crude energy (kcal g^{-1})] /No. of days

VIII. Calculation of biological indices

$$\text{Condition factor (k)} = \frac{\text{Total body weight (g)}}{\text{Length (cm)}^3} \times 100$$

$$\text{Gastro-somatic index} = \frac{\text{Total weight of gut (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{Liver-somatic index} = \frac{\text{Total weight of liver (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{Gonado-somatic index} = \frac{\text{Total weight of gonad (g)}}{\text{Total body weight (g)}} \times 100$$

IX. Statistical Analyses

The data were statistically analysed using standard methods (Snedecor and Cochran, 1967). All analyses were made on Casio fx-451 M.

Table 1. Composition of mineral mixture*

Minerals	g/100 g
Calcium biphosphate	13.48
Calcium lactate	32.40
Ferric citrate	02.97
Magnesium sulfate	13.70
Potassium phosphate (Dibasic)	23.86
Sodium biphosphate	08.72
Sodium chloride	04.35
Aluminium chloride.$6H_2O$	00.015
Potassium iodide	00.015
Cuprous chloride	00.010
Manganous sulfate.H_2O	00.080
Cobalt chloride.$6H_2O$	00.100
Zinc sulfate.$7H_2O$	00.300

* Halver (1989).

Table 2. Composition of vitamin mixture*

Vitamins	g/100 g
Choline chloride	0.500
Inositol	0.200
Ascorbic acid	0.100
Niacin	0.075
Calcium pantothenate	0.050
Riboflavin	0.020
Menadione	0.004
Pyridoxine.HCl	0.005
Thiamin	0.005
Folic acid	0.0015
Biotin	0.0005
α-tocopherol acetate	0.040
Vitamin B_{12} (10 mg/500 ml H_2O)	0.00001 (0.5 ml)

* Halver (1989)

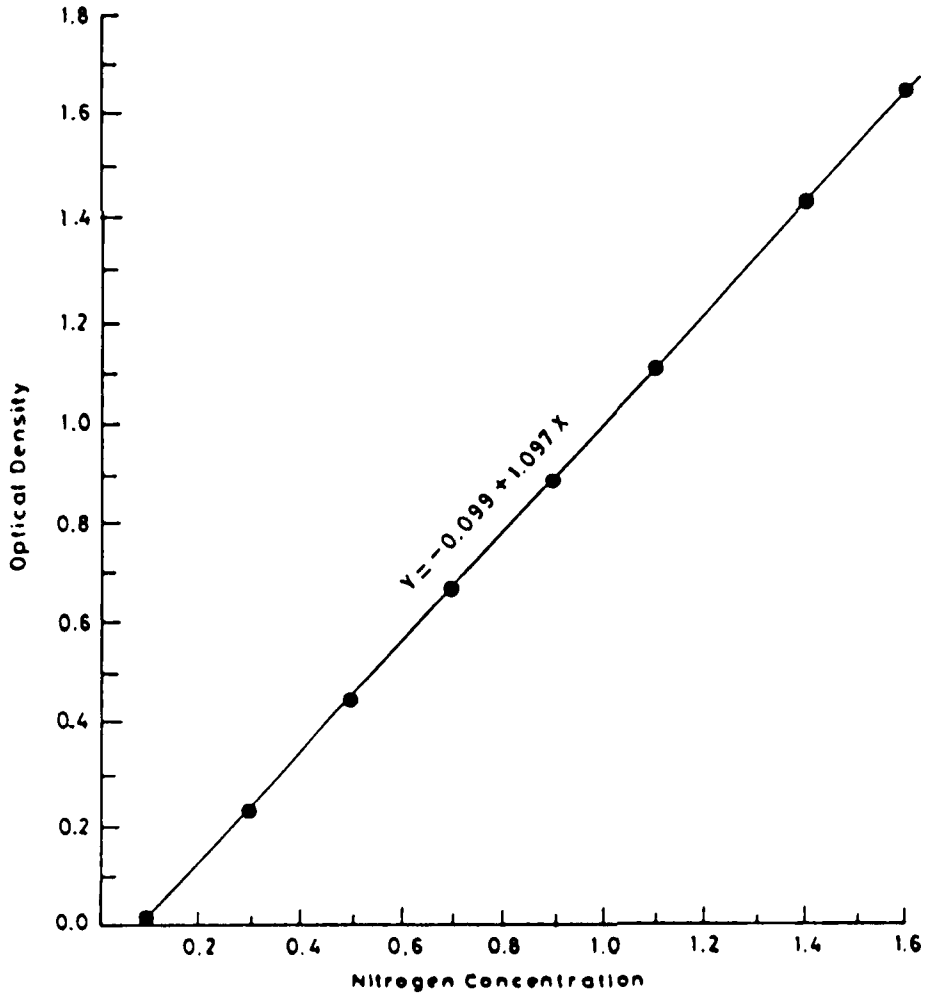

Fig. 1. Calibration curve of nitrogen.

PART I

CHAPTER 1

OPTIMUM FEEDING RATE, AND ENERGY AND PROTEIN MAINTENANCE REQUIREMENTS OF YOUNG CLARIAS BATRACHUS (LINNAEUS), A CULTIVABLE CATFISH SPECIES*

INTRODUCTION

Although polyculture of major carps has been well established in India, aquaculture of catfishes is yet to be commercialised. Culture of Clarias batrachus, a highly valued omnivorous catfish, is gaining importance in view of its compatability with other cultivable fish species and the development of its induced breeding technique. The need for the development of artificial diet for this species has been emphasized (Tripathi, 1991) but lack of information on its nutrition and thereby non-availability of artificial diet becomes a major limiting factor for its intensive aquaculture.

Information on the optimum feeding rate of cultured fish is important in achieving efficient production. Since feeding rate affects the nutrient requirements, a knowledge of optimum feeding rate is considered a prerequisite to nutrient requirement determinations (Tacon and Cowey, 1985 and Talbot, 1985). Ration level is also reported to influence fish growth, feed conversion and chemical composition (Huisman, 1976; Reddy and Katre, 1979; Reinitz, 1983[ab]).

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*Accepted for publication in Aquacult. Fish. Manage.

Several factors, including ration size and quality, size of the fish and water temperature (Brett, 1979) influence optimum feed requirement. In commercial practice, although control of environmental temperature may not be practicable, ration size can be manipulated to maximise production.

The present study deals with the effect of feeding rates on the growth and body composition of C. batrachus, leading to the estimation of its optimum ration level, and protein and energy maintenance requirements. The information generated will be of interest both to fish farmers and nutritionists.

MATERIALS AND METHODS

Preparation of experimental diet

Casein-gelatin based semipurified (40% C.P., 355.3 kcal/100g) diet was used for the study (Table 1). Method of preparation of the diet has been described under General Methodology Section (page - 13). As a prophylactic measure, oxytetracycline (Pfizer, 30 mg/100 g diet; as fed) was added to the diet. Since digestible or metabolizable energy value for the ingredients used are not available for C. batrachus, physiological fuel values, 3.5, 4.5 and 8.5 kcal/g for carbohydrate, protein and lipid, respectively (Jauncey, 1982[a]), were utilized to calculate the energy content of the diet. An energy to protein ratio of 8.8 (kcal g^{-1}) found optimum for this species (Chapter 5), was maintained in the diet.

Feeding trial

Source of fish, their acclimation, and details of general experimental design have been given elsewhere (page12-14).

For each dietary treatment, fish (12.40 ± 0.4 cm; total length and 10.95±0.14 g) were stocked randomly, in triplicate group of five fish each, in polyvinyl circular trough (water volume 55 l; exchange rate 1 l/min). Water temperature over the experimental period was 30±2°C.

Fish were fed ration levels 0 to 8% body weight per day ($Bw.day^{-1}$), dry to wet weight basis, twice daily for 5 weeks. Mass weight was taken weekly and the amount of ration readjusted for subsequent feeding. Initially, no unconsumed feed was noticed in the experimental trough, but during the later part of the experiment fish required longer time to consume their daily feed allowance, with some feed found accumulating in higher ration groups. However, no estimate of uneaten feed, if any, was made. Survival during the trial was 100%.

Assuming that metabolic expenditure in fish is related to their body weight, Winberg (1956) and others have expressed the consumption with the equation $T = \alpha W^{\beta}$ (where, T stands for intake, W for weight, α and β are the parameters referred as "metabolic level" and "weight exponent", respectively), the value of β being 0.8 (Hogendoorn, 1983). For calculating the maintenance requirements of C. batrachus, therefore, the ration level has been expressed in

terms of $g/W^{0.8}$ for protein and $kcal/W^{0.8}$ for energy per day, while the growth increment measured in terms of $g/W^{0.8}$ per day. Other parameters are given without considering the metabolic weight.

Growth parameters and utilization efficiencies were measured using standard definitions (page 19-21).

Gross energy and proximate analyses

Gross energy and proximate composition of fish tissue were analysed according to standard methods (page 14-18). Required number of acclimated fish were taken out for the assessment of initial body composition. The fish were killed with an overdose of MS-222 (Ayerst Laboratories, New York, USA), skinned and the trunk muscle scraped and homogenised. At the end of the feeding trial, three fish from each trough were randomly taken out and analysed for their final gross energy and proximate composition.

Statistical analyses

The data was statistically analysed and best fit line obtained with least square method for daily average growth increment, net protein and energy gains against dietary protein and energy intakes. A second degree polynomial curve was obtained, using specific growth rate (SGR) as dependent variable, to quantify the optimum ration level. Test of one-way analysis of variance was made to examine significant change of means over the initial (Sokal and Rohlf, 1981). Comparison between treatment means were also

made using Duncan's (1955) multiple range test. Coefficient of variation (CV) was calculated for food conversion ratio (FCR) to determine variation within the treatments (Sokal and Rohlf, 1981). The level of significance was tested at 95% confidence limit.

RESULTS

Results of 5-week feeding trial have been given in Table 2-4 . Feeding fish with varying ration levels produced a linear ($r = 0.985$) increase in average daily growth increment (Fig. 1-2) up to 4% (Bw. day^{-1}), corresponding to 6.03 g protein/($kg^{0.8}$ $Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}Bw.day^{-1}$). Within the linear growth portion, average daily growth increments of fish (Y) over increasing levels of protein and energy (X) was described by the equations $Y = -2.13 + 2.26\ X$ for protein, and $Y = -2.21 + 0.2568\ X$ for energy. Maintenance requirements of 0.942 g/($kg^{0.8}Bw.day^{-1}$) for protein and 8.605 kcal/($kg^{0.8}Bw.day^{-1}$) for energy were obtained from these equations. Weight gains beyond 6.03 g protein/($kg^{0.8}Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}Bw.day^{-1}$) intakes were insignificant. Starved fish continued to lose weight.

Net gains in muscle protein and energy also exhibited linear increase ($r = 0.975$) with increasing levels of feeding up to 6.03 g protein/($kg^{0.8}Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}Bw.day^{-1}$), and these have been described by the equations $Y = -0.5175 + 0.5146\ X$ for protein and $Y = -0.625 + 0.0621\ X$ for energy (Fig. 1-2). The above equations were also used to estimate protein and energy

maintenance requirements, the former being 1.005 g/($kg^{0.8}$Bw.day^{-1}) and the latter 10.06 kcal/($kg^{0.8}$Bw.day^{-1}). Increasing protein and energy intakes above 6.03 g protein/($kg^{0.8}$Bw.day^{-1}) and 53.58 kcal energy/($kg^{0.8}$Bw.day^{-1}) did not result in increased net gains in body protein or energy. Starved fish showed net loss of both protein and energy.

Although highest gains in body protein and energy were obtained by feeding fish with 6.03 g protein/($kg^{0.8}$Bw.day^{-1}) and 53.58 kcal energy/($kg^{0.8}$Bw.day^{-1}), maximum gross growth efficiency, and protein and energy conversion efficiencies occurred at 4.49 g protein/($kg^{0.8}$Bw.day^{-1}) and 39.91 kcal energy/($kg^{0.8}$Bw.day^{-1}).

The best FCR was obtained in the group fed 4.49 g protein/($kg^{0.8}$Bw.day^{-1}) and 39.91 kcal energy/($kg^{0.8}$Bw.day^{-1}), and were poor above or below this feeding level. FCR plotted against ration levels produced a typical U-shaped curve (Fig. 3). CV for FCR were lower in fish fed at or near 3% (Bw.day^{-1}) and higher in those fed above or below this level of feeding.

The proximate composition of fish fed varying ration levels has been given in Table 4. The body composition varied with levels of feeding. Moisture and lipid were significantly affected while changes in protein and ash contents were insignificant. With increase in feeding rate from 1 to 4% (Bw.day^{-1}), moisture percentage decreased whereas crude fat registered an increasing trend. In starved fish, however, compared to protein, crude fat was greatly reduced whereas moisture and ash contents increased markedly.

DISCUSSION

The data indicate that, over the experimental period, C. batrachus fed at 1% (Bw.day^{-1}) increased its live weight only to 2.13 g/kg$^{0.8}$day^{-1} whereas the group fed 4% (Bw.day^{-1}) increased to 10.49 g/kg$^{0.8}$ day^{-1}. No significant growth improvement was noticeable at a ration level above 4% (Bw.day^{-1}). It appears that, between a feeding rate of 3 to 4%, a larger proportion of dietary nutrients was utilized by the fish for its growth as reflected by feed/nutrient conversion values. The lower rate of daily average weight increment and poor FCR in fish fed 1% (Bw.day^{-1}) suggest that this ration level approximates only the maintenance requirement for nutrients, whereby a major portion of the ingested nutrient gets utilized to maintain life, with only a small proportion becoming available for growth. Growth pattern of C. batrachus, within submaximal ration, was similar to that reported for several other fish species (Huisman, 1976; Kaushik and Luquet, 1984; Gatlin et al., 1986; Hung and Lutes, 1987; Storebakken and Austreng, 1987[b]).

Growth increments of C. batrachus, fed graded levels of ration over the period of feeding trial, when regressed indicates the maintenance requirements of this species to be 0.942 g protein/(kg$^{0.8}$Bw.day^{-1}) and 8.6 kcal energy/(kg$^{0.8}$Bw.day^{-1}). Regressing body protein and energy changes back to zero, the values approximated as 1.005 g protein/(kg$^{0.8}$Bw.day^{-1}) and 10.06 kcal /(kg$^{0.8}$Bw.day^{-1}). In both ways, the estimated maintenance requirements of this fish for protein and energy seem comparable.

This strengthens the fact that feeding graded ration to fish and regressing the gain to zero can be employed as a convenient and useful method for assessing the maintenance requirement. Earlier workers (Kaushik et al., 1981; Gatlin et al., 1986; Storebakken and Austreng, 1987[b]; Hung and Lutes, 1987; Brown et al., 1990), with the exception of Huisman (1976) and few others, reported the maintenance requirements of fish without considering their metabolic weight. Besides body weight (metabolic), requirements of fish have also been stated to be proportional to water temperature (Smith et al., 1978). Huisman (1976), while estimating the maintenance ration per week, reported the values as 172 kcal/$kg^{0.8}$ for carp (average weight 40.98 g) at 23°C and 120 kcal/$kg^{0.8}$ for trout (average weight 68.4 g) at 15°C. Using regression analysis, Gatlin et al. (1986) worked out the maintenance requirements for protein (1.0-1.32 g/kg Bw. day^{-1}) and energy (15.06-17.33 kcal/kg.Bw. day^{-1}) in channel catfish reared at 26.7°C. Maintenance requirement for protein in juvenile sturgeon (20-40 g weight), as calculated from Huang and Lutes (1987), comes to 2.5 g/(kg.Bw.day^{-1}) at 20°C. Feeding high protein diet to juvenile channel catfish (average weight 9.0 g) reared at 24-26°C, Brown et al. (1990) estimated its maintenance energy needs to be 23.7 kcal/(kg.Bw.day^{-1}).

The typical U-shaped curve of FCR in C. batrachus points towards an optimum (3%) feeding rate for this species. Reddy and Katre (1979) observed better conversion efficiency in the catfish, Heteropneustes fossilis, at a similar ration level. Poorer FCR

observed during the study at higher ration levels may be due to some loss of nutrient and wastage of food, since fish took longer time to consume their daily feed allowance to reach satiety. Vens-Cappell (1978) attributed poor FCR at higher levels of ration in rainbow trout to reduced protein digestibility.

High CV for FCR at the lowest (1%) feeding rate may be the result of the fish receiving ration closer to their maintenance (0.51-0.62%, calculated) level. A slight difference in food consumption produced a differential growth leading to greater variations in FCR. Similarly, high CV in fish fed 5-8%($Bw.day^{-1}$) may be due to the realization of full growth potential of the individual fed above the optimum level. Minimum CV at 3% ($Bw.day^{-1}$) also suggests this as the optimum feeding rate for C. batrachus. On the basis of similar pattern of changes, Hung and Lutes (1987) obtained the optimum feeding level for white sturgeon.

Optimization of ration level for C. batrachus at 3% ($Bw.day^{-1}$) further becomes strengthened on drawing a tangent from the origin over the ration level and SGR relationship curve (Fig. 4; second degree polynomial). Several workers (Zeitoun et al., 1976; Teng et al., 1978; Reddy and Katre, 1979; De Silva et al., 1989; De Silva and Gunasekera, 1991) in the past have used second degree polynomial regression for nutrient optimization and considered this as statistically more valid method. With similar method, Huisman (1976) determined the optimum food ration for carp and trout as 2.2 and 1.3 (% $Bw.day^{-1}$), respectively. Using breakpoint analysis (Duncan's multiple range test), Hung and Lutes (1987) reported

2.0% ($Bw.day^{-1}$) as optimum for juvenile sturgeon.

The maximum gross growth efficiency in C. batrachus, coinciding with its optimum 3% ($Bw.day^{-1}$) ration level (Table 2 , Fig. 4), can be taken as characteristic of the relationship between these two parameters.

Feed conversion and protein utilization in fish are influenced, besides other factors, by the biological value of feed and its nutrient ratios, as also by the maintenance requirements of the species concerned (Brett and Groves, 1979). Since amount of feed lost in any aquatic system is difficult to estimate precisely, a limitation is imposed on the comparison of feed/nutrient conversion estimates obtained for one set of experiment with the other. However, increase in gross growth efficiency, as well as protein and energy conversion efficiencies, in C. batrachus up to 4.49 g protein/($kg^{0.8}Bw.day^{-1}$) and 39.91 kcal energy/($kg^{0.8}Bw.day^{-1}$), and a gradual decline at higher levels of ration, indicate efficient nutrient utilization by the fish up to the aforesaid levels. Feeding fish beyond these levels is a wastage. The observed conversion efficiency values for the fish, which conform with the findings on other fishes (Brett and Groves, 1979; Henken et al., 1987), qualify the fish as an efficient converter of dietary protein and energy.

The results on C. batrachus also indicate that body composition of fish is affected by varying rates of feeding. Increased feeding rate progressively alters body moisture, fat, protein and ash contents up to a ration level of 4% ($Bw.day^{-1}$). At higher

feeding rates, however, no specific pattern could be noted. The changes were comparable to the results obtained by other workers (Huisman, 1976; Gatlin et al., 1986). While no significant changes were noted for protein and ash contents, moisture and fat were significantly influenced by the ration levels. This seems consistent with the observation on juvenile white sturgeon (Hung and Lutes, 1987[b]). The findings of Storebakken and Austreng (1987) were also similar to the results on C. batrachus, except for the change in body ash.

The proximate composition of fish is known to be affected by two major factors : stage of development and diet. In general, changes in level of dietary nutrients do not affect body protein and ash since their levels in body tissue are specified by genetic code (Buckley and Groves, 1979). Nevertheless, in the present study when fish were starved, the amount of moisture and ash increased, whereas, a sizeable decrease in fat and a slight decrease in protein percentage occurred over their initial values. Similar changes were reported in other fishes (Weatherly and Gills, 1981; Reinitz, 1983[ab]; Hogendoorn, 1983). Further, a net loss of energy noted in starving C. batrachus indicates that, although both lipid and protein get metabolized during the starvation, there is a preferential catabolism of body fat stores. Fish fed at the lowest (1% Bw.day^{-1}) ration level contained a slightly lower percent of body fat, though at the same ration level they could manage to gain some amount of protein over their initial, suggesting that in this fish body fat is the

preferred energy reserve, over protein, for deposition and/or mobilization. A corollary to this finding was apparent in other fish species as well (Hogendoorn, 1983; Hung and Lutes, 1987; and Brown et al., 1990).

SUMMARY

Protein and energy maintenance, and optimum feed requirements are reported in the catfish, C. batrachus, fed purified diet (40% C.P.; 355.3 kcal/100 g) at 0 to 8% ($Bw.day^{-1}$) ration levels. Linear ($r = 0.985$) increase was observed in daily average growth increment up to ration level of 4% ($Bw.day^{-1}$), corresponding to 6.03 g protein/($kg^{0.8}Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}Bw.day^{-1}$). Maintenance requirements, obtained by regressing daily average growth increment to zero, were 0.942 g/($kg^{0.8}Bw.day^{-1}$) for protein and 8.605 kcal/($kg^{0.8}Bw.day^{-1}$) for energy. Net gains in muscle protein and energy also depicted linear increase ($r = 0.975$) with feeding levels up to 6.03 g protein/($kg^{0.8}Bw.day^{-1}$) and 53.58 kcal energy/($kg^{0.8}Bw.day^{-1}$). On fitting the above data to regression equation, the amount of dietary protein and energy required to maintain a constant amount of body protein and energy, values of 1.005 g/($kg^{0.8}Bw.day^{-1}$) and 10.06 kcal/($kg^{0.8}Bw.day^{-1}$) were obtained for protein and energy, respectively. The optimum feeding rate for this species, as evident from specific growth rate and conversion efficiencies, appear to be 3% ($Bw.day^{-1}$) at 30±2°C. Moisture and lipid contents of muscle were found significantly ($P < 0.05$) affected by the ration levels.

Table 1. Ingredient and proximate composition of experimental diet

Ingredients	g/100 g (as fed)
Casein (vitamin-free; 84.6% C.P.)[1]	35.71
Gelatin (87.0% C.P.)[2]	11.41
Dextrin	30.66
α-cellulose	08.22
Oil mix (2:1 corn and cod liver oil)	08.00
Vitamin mix	01.00
Mineral mix	03.00
Carboxymethyl cellulose	02.00
Proximate composition % (calculated) :	
Crude protein	40.00
Crude fat	08.00
Carbohydrate	31.00
Energy (kcal/100 g)	335.30
E/P ratio (kcal/g)	08.80

[1]ICN Pharmaceuticals, Cleveland, Ohio, USA; [2]Loba Chemi, India

Table 2. Total diet fed, initial and final average fish weight, growth increment per fish and gross growth efficiency of C. batrachus fed varying levels of experimental diet

Feeding rate			Total diet fed (g)	Initial average weight (g)	Final average weight (g)	Growth increment $g/kg^{0.8}day^{-1}$	Gross growth efficiency (%)
%($Bw.day^{-1}$)	Energy kcal $/kg^{0.8}$ $Bw.day^{-1}$	Protein $g/kg^{0.8}$ $Bw.day^{-1}$					
0.0	00.00	0.00	000.00	20.56±0.03	16.21±0.04	$-3.03±0.52^{c}$	-
1.0	13.85	1.56	096.48	20.23±0.02	23.75±0.42	$2.13±0.21^{d}$	$54.59±5.69^{b}$
3.0	39.91	4.49	358.65	20.52±0.04	39.97±0.34	$9.13±0.14^{ab}$	$81.38±2.07^{c}$
4.0	53.58	6.03	496.35	19.87±0.00	42.90±0.12	$10.49±0.02^{b}$	$69.59±0.39^{d}$
5.0	68.39	7.70	597.24	19.92±0.04	38.48±0.26	$8.97±0.02^{ab}$	$46.60±0.33^{e}$
6.0	80.52	9.06	679.41	19.40±0.00	36.45±0.05	$8.53±0.02^{a}$	$37.69±0.88^{a}$
6.5	87.82	9.89	759.15	19.51±0.05	38.12±1.32	$9.08±0.44^{ab}$	$36.72±2.25^{a}$
7.0	96.08	10.82	806.49	19.78±0.33	36.78±1.74	$9.05±0.86^{ab}$	$33.24±3.03^{a}$
8.0	111.64	12.57	970.02	19.76±0.01	38.19±1.47	$8.95±0.52^{ab}$	$28.40±1.73^{a}$

Values are means ± SE of triplicate fish groups. Means in each column with same superscript are insignificantly different ($P<0.01$)

Table 3. Conversion efficiencies and specific rate of change for protein and energy in C. batrachus fed varying levels of experimental diet

	Feeding rate		Conversion efficiency (%)		Specific rate of change (% day^{-1})	
%(Bw.day^{-1})	Energy kcal/($kg^{0.8}$ Bw.day^{-1})	Protein g/($kg^{0.8}$ Bw.day^{-1})	Protein	Energy	Protein	Energy
0.00	00.00	00.00	00.00	00.00	-0.73 ± 0.11^{a}	-0.84 ± 0.10^{a}
1.00	13.85	01.56	22.50 ± 010^{a}	16.39 ± 0.08^{a}	0.47 ± 0.07^{b}	0.44 ± 0.07^{b}
3.00	39.91	04.49	36.47 ± 0.06^{c}	28.83 ± 0.16^{c}	2.07 ± 0.23^{c}	2.09 ± 0.10^{c}
4.00	53.58	06.03	30.63 ± 0.07^{d}	25.67 ± 0.03^{d}	2.33 ± 0.22^{c}	2.47 ± 0.21^{c}
5.00	68.39	07.70	20.60 ± 0.02^{a}	15.70 ± 0.01^{a}	2.02 ± 0.17^{c}	1.99 ± 0.16^{c}
6.00	80.52	09.06	15.83 ± 0.19^{ab}	13.69 ± 0.11^{ab}	1.88 ± 0.10^{c}	2.01 ± 0.11^{c}
6.50	87.82	09.89	16.61 ± 0.02^{ab}	13.81 ± 0.07^{ab}	2.10 ± 0.13^{c}	2.18 ± 0.15^{c}
7.00	96.08	10.82	13.80 ± 0.21^{b}	11.41 ± 0.17^{b}	1.97 ± 0.16^{c}	2.05 ± 0.16^{c}
8.00	111.64	12.57	12.46 ± 0.13^{b}	10.39 ± 0.23^{b}	2.02 ± 0.23^{c}	2.11 ± 0.13^{c}

Values are means ± SE of triplicate fish groups. Means in each column with same superscript are insignificantly different ($P<0.01$)

Table 4. Proximate composition and energy content in the muscle tissue of C. batrachus fed varying levels of experimental diet

Feeding rate			Proximate composition (g/100 g, dry matter)				kcal g^{-1} dry matter
%(Bw.day^{-1})	Energy kcal/($kg^{0.8}$ Bw.day^{-1})	Protein g/($kg^{0.8}$ Bw.day^{-1})	Moisture (g/100 g, wet weight)	Crude protein	Crude fat	Ash	
0.00	00.00	00.00	80.48±0.07^{a}	79.67±0.64	08.05±0.61^{a}	7.05±0.06	5.34±0.03
1.00	13.85	01.65	79.90±0.43^{b}	79.30±0.90	09.98±0.65^{b}	6.70±0.14	5.47±0.02
3.00	39.91	04.49	78.69±0.25^{c}	79.15±0.80	13.08±0.52^{cd}	6.03±0.09	5.54±0.02
4.00	53.58	06.03	78.50±0.34^{c}	78.10±0.71	14.87±0.85^{c}	5.80±0.02	5.70±0.04
5.00	68.39	07.70	78.67±0.45^{c}	78.47±0.67	12.65±0.79^{cd}	5.81±0.08	5.63±0.01
6.00	80.52	09.00	78.77±0.25^{c}	77.19±0.61	13.89±0.73^{cd}	6.01±0.02	5.70±0.06
6.50	87.82	09.89	78.19±0.42^{c}	77.92±0.88	13.24±0.85^{cd}	5.98±0.08	5.60±0.04
7.00	96.08	10.82	78.24±0.50^{c}	78.26±0.79	12.39±1.14^{c}	6.18±0.08	5.63±0.02
8.00	111.64	12.57	78.00±0.29^{c}	78.41±1.28	12.54±0.82^{cd}	6.10±0.08	5.63±0.02
Initial sample			79.75±0.32	78.24±0.55	11.13±1.01	6.68±0.00	5.46±0.03

Values are means ± SE of triplicate fish groups. Means in each column with same superscript are insignificantly different ($P < 0.05$)

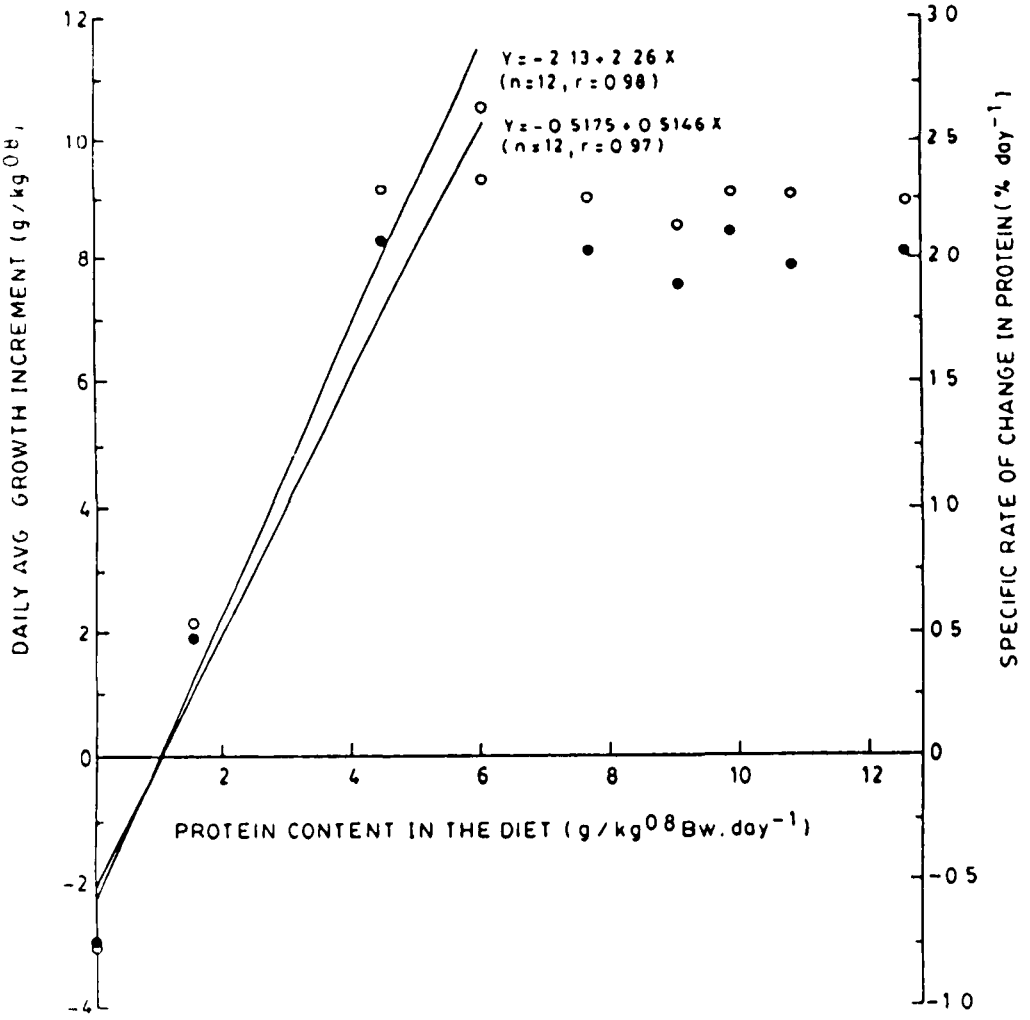

Fig. 1. Daily average growth increment (o) and rate of change in body protein (●) of C. batrachus fed varying levels ($g/kg^{0.8}$ Bw.day^{-1}) of dietary protein.

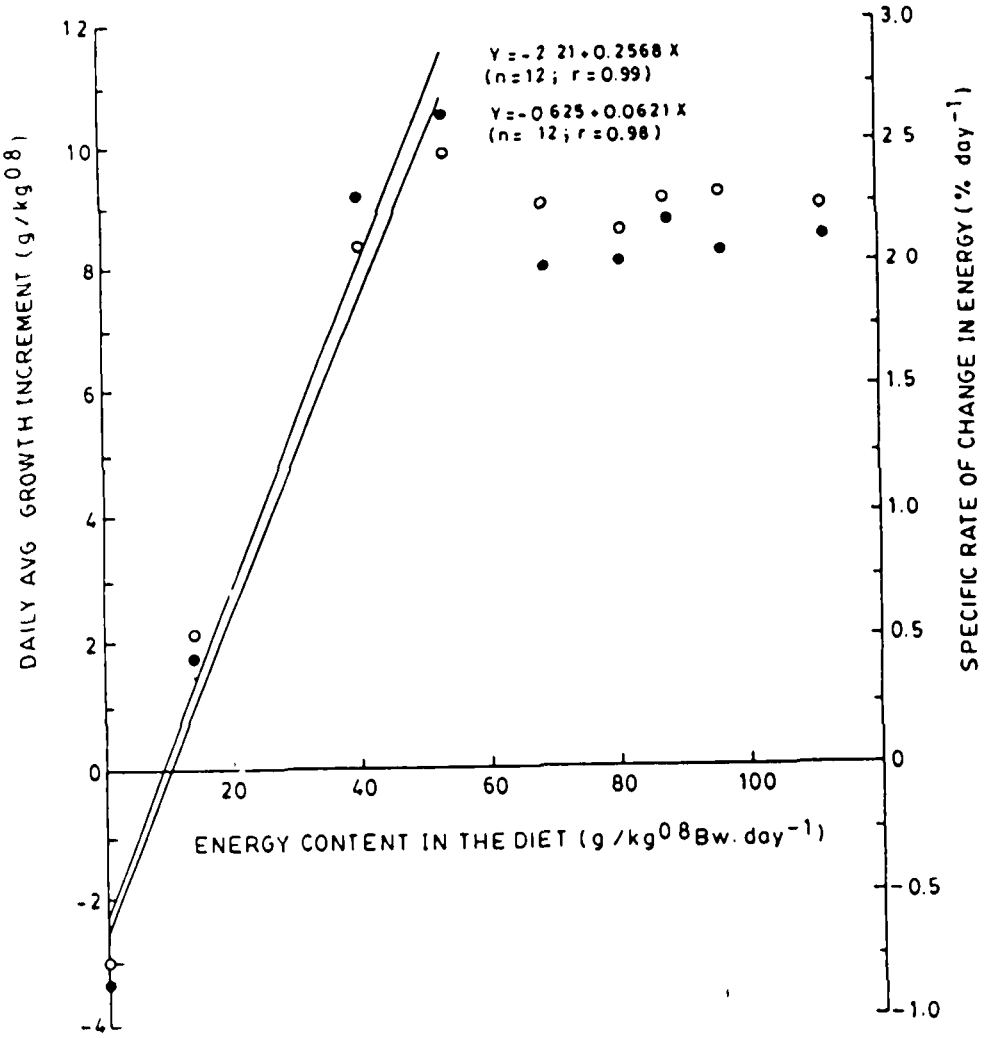

Fig. 2. Daily average growth increment (●) and rate of changes in body energy (o) of C. batrachus fed varying levels (kcal/$kg^{0.8}$ Bw.day^{-1}) of dietary energy.

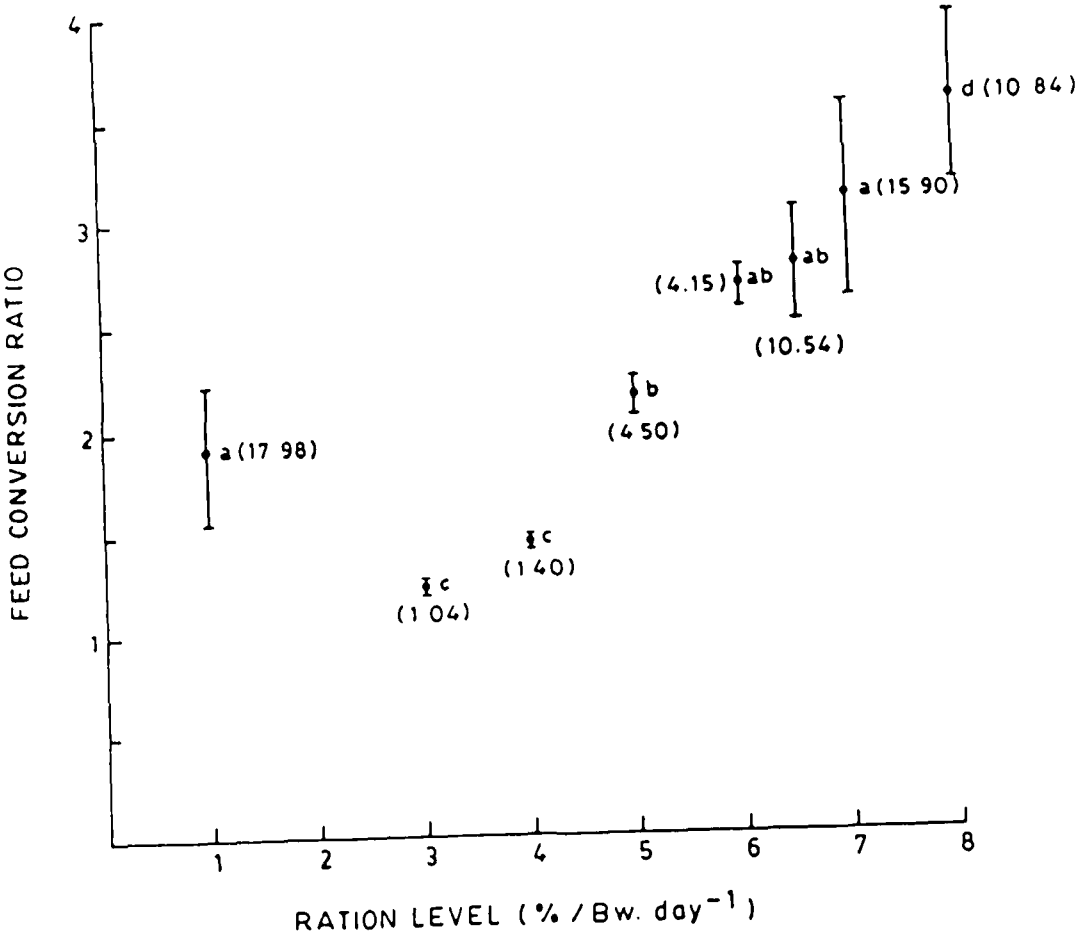

Fig. 3. Effect of ration levels (% Bw.day $^{-1}$) on food conversion ratio in C. batrachus. Each point represents mean ± SE of triplicate fish groups. Means with same superscript are insignificantly different ($P < 0.01$). Values in parentheses represent the coefficient of variation.

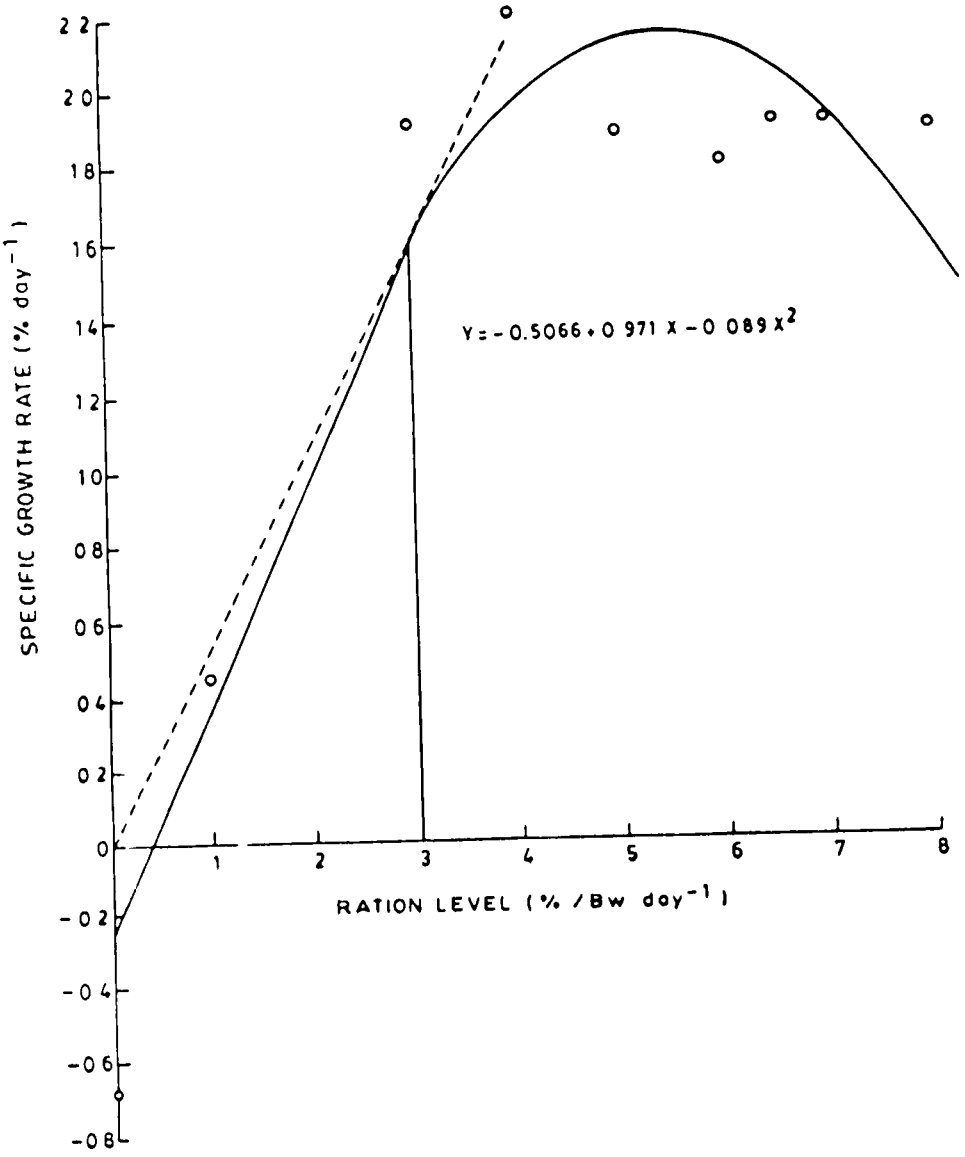

Fig. 4. The second degree polynomial relation of specific growth rate (% day $^{-1}$) and ration level (%/Bw.day $^{-1}$) in C. batrachus.

CHAPTER 2

ENERGY DIGESTIBILITY COEFFICIENT OF COMMONLY USED FEEDSTUFFS IN DIFFERENT SIZE-CLASSES OF INDIAN MAJOR CARPS, LABEO ROHITA (HAMILTON) AND CIRRHINUS MRIGALA (HAMILTON)

INTRODUCTION

Diet development for practical feeding requires a knowledge of nutritional requirements, and the ability of the concerned species to digest and absorb the nutrients from feed ingredients. Energy digestion coefficients of various feedstuffs have been reported for several fish species (Cruz, 1975; Stickney and Lovell, 1977; NRC, 1981; Cho et al., 1982; Popma, 1982; Wilson and Poe, 1985; Hanley, 1987; and Hossain and Jauncey, 1989) using direct or indirect methods. Information on the digestibility of feed ingredients used for feeding Indian major carps, in traditional or semi-intensive farming system, is limited (Singh, 1992). In order to meet their nutrient/energy requirement through practical feeding, and to replace one ingredient with the other for economic reasons, knowledge of digestibility coefficient for such feedstuffs is essential.

In fish, the nutrient/energy digestibility, for a particular ingredient, is known to be species specific. Several other factors, including age/size of the fish, also govern the extent of digestibility (Hastings, 1969; Windell et al., 1978). This study analyses the energy digestibility coefficient for some commonly used

feedstuffs in two size-classes of Indian major carps, namely Labeo rohita and Cirrhinus mrigala.

MATERIALS AND METHODS

Experimental fish and acclimation

Hatchery produced fingerling of L. rohita and C. mrigala were used for the study (page 12). For each species, two size-groups were sorted out, stocked in 70 l flow-through type circular trough (water volume, 55 l and exchange rate, 1 l/min), and acclimated to experimental condition. Details of the fish size and stocking density are given in Table 1.

Feedstuffs

Nine different feedstuffs, generally used by fish farmers in various parts of India for the supplementary feeding of carps, were selected for the study. Mixed species of trash fish purchased from the local market were cooked and pressed, and dried (60°C) in hot air oven for the preparation of fish meal. Slaughter house waste (buffalow intestine) was collected, cut open and cleaned with a strong jet of water. It was then processed in the above manner. Dried silk worm (Bombyx mori) pupae were procured from a farmer in West Bengal, while expeller type rocket salad oil cake, mustard oil cake, groundnut oil cake, and rice polish were obtained from local market. Soybean oil cake (with hull, solvent extracted) was supplied by Parag Oil Mill, Aligarh. The feedstuffs were finely

powdered and their gross energy and proximate composition estimated (Table 2) using the methods described under General Methodology section (page 14-18).

Preparation of experimental diets

The experimental diets were formulated following the methods adopted by Cruz (1975) and Popma (1982). A mixture of α-cellulose and finely sieved sugarcane bagasse (1:1) was used in various quantities to maintain a uniform (15-16%) amount of dietary fibre to facilitate a homogeneous enzyme action. The lipid level in the diet was maintained at 9-10% by adding an exogenous oil mixture (2:1, corn and cod liver oil). Gelatin and carboxymethyl cellulose were used as binders while chromic oxide was employed as indigestible marker (Table 3).

Method of preparation of the experimental diets was the same as described under General Methodology section (page 13), except that dry dietary ingredients were thoroughly mixed together in a mixer before adding these to the cooking bowl containing gelatin in the dissolved state.

Digestibility trial

Water exchange rate in fish holding troughs throughout the trial was maintained at 1 l/min. Care was taken to ensure that fecal matter does not escape through the outlet. For acclimation to experimental diets, fish were fed ad lib daily at 0900 and 1600

h for 7 days. Unconsumed food was siphoned off 2 h after each feeding. After acclimation, fecal matter was collected for digestibility estimates daily at 0800 and 2200 h, and this continued till enough sample was collected. Fecal collection was made over a fine mesh strainer through slow siphoning, using a narrow (8.0 mm) plastic tube, to avoid breakage of thin fecal strings and minimize nutrient loss which is inherent with this method. The collections were pooled together in a plastic container and stored (-20°C) for analysis. Each experiment was carried out in triplicate. The water temperature ranged between 24-28°C. After each run, the troughs were thoroughly cleaned by scrubbing and rinsed with dilute $KMnO_4$ solution. Fishes were also given a prophylactic dip in 4-5 ppm $KMnO_4$ solution. Before feeding the next experimental diet, fishes were deprived of food for 3-4 days.

Analytical methods

Prior to their inclusion, the feedstuffs were analysed for their proximate composition using standard methods (page 14-18). Gross energy of feedstuffs, diet and feces were estimated on ballistic (adiabatic) bomb calorimeter (page 18). Method of estimation of chromic oxide in the sample is given under General Methodology section (page 18-19).

Digestible energy content in each diet was calculated with the following formula (Page and Andrews, 1973):

$$\text{Digestible energy (kcal g}^{-1}) = Ed-(E_f \times \frac{I_i}{I_f})$$

where,

Ed = gross energy of diet (kcal g^{-1}),

E_f = gross energy of feces (kcal g^{-1}),

I = concentration of indicator (absorbance),

i = ingesta and

f = feces.

Digestible energy values of various diets were fitted to the following equation to estimate the digestibility coefficients for each feed ingredient :

$$\begin{matrix}\text{(a)}\\ \text{Digestible energy}\\ \text{of the diet}\\ (\text{kcal } g^{-1})\end{matrix} = \begin{bmatrix}\text{(b)}\\ \text{Gross energy (kcal } g^{-1})\\ \text{of test ingredient}\end{bmatrix}\begin{bmatrix}\text{(x)}\\ \text{Digestible energy}\\ (\text{kcal } g^{-1}) \text{ of test}\\ \text{ingredient (\%)}\end{bmatrix} + \begin{bmatrix}\text{(c)}\\ \text{Gross energy (kcal } g^{-1})\\ \text{of oil supplement}\end{bmatrix}\begin{bmatrix}\text{(d)}\\ \text{Digestible energy}\\ (\text{kcal } g^{-1}) \text{ of oil}\\ \text{supplement (\%)}\end{bmatrix}$$

where, $x = (a-cd)/b$

The components of the formulated diet, besides the test ingredient, which contributed to its gross energy were gelatin, oil mix, and fibre (mixture of α-cellulose and sugarcane bagasse). Since gelatin formed only 1% of the diet, its energy value was not taken into account while calculating the digestibility coefficient. Oil mix used was considered approximately 90% digestible, as reported for several other herbivore and omnivore fish species (Takeuchi, 1979; NRC, 1983; and Hanley, 1987).

Statistical analyses

The data on energy digestibility in each size-class of the two species were subjected to one-way analysis of variance to test the variability. Student's t-test was employed for test of significance (Snedecor and Cochran, 1967). Significant differences between means were compared using Duncan's (1955) multiple range test.

RESULTS

The data (Table 4) indicates that, within each size-class of the test species, L. rohita and C. mrigala, digestibility varies ($P < 0.01$) with feedstuffs. However, no significant ($P < 0.01$) difference in digestibility of the feedstuffs tested was noticed between different size-classes of the two species. Both the species exhibited poor digestibility for rice polish (Oryza sativa), while soybean oil cake (Glycine max) was found to be the most digestible ingredient among the various feedstuffs. Rocket salad (Eruca sativa) oil cake, mustard (Brassica campestris) oil cake, wheat bran (Triticum aestivum), fish meal, slaughter house waste, and dried silk worm pupae gave comparable values ($P < 0.01$) for both the size-classes of the two fishes. When animal origin feedstuffs were compared, coefficients of digestibility did not differ much. Within each size-class, in both the species, the mean digestibility values between plant and animal origin feedstuffs were insignificantly ($P < 0.01$) different. Categorywise, digestibility of plant and animal origin feedstuffs, compared between size-classes, showed no

significant ($P < 0.01$) difference, in both the species. With the exception of animal origin feedstuffs in larger size-class, differences in digestibility of feedstuffs, irrespective of source, were insignificant ($P < 0.01$) between the two species for both the size groups.

DISCUSSION

The variations in the energy digestibility, observed amongst the feedstuffs, may be attributed to the inherent complexity of energy nutrients, particularly carbohydrate and protein, as also to the enzyme (digestive) profile of the fish concerned. Since a constant fibre content was maintained in the test diets, such variations could not be due to crude fibre level of the diet. However, the possibility that fibrous (cellulose, hemicellulose and lignin) nature of the ingredient itself could cause variations in digestibility can not be ruled out. Lowest digestibility noted for rice polish in both the fish species indicate that its inclusion in the diet may not serve as an energy source, but rich inorganic content of this ingredient may improve the nutritional quality of the diet. Law (1984, 1986) has reported poor digestibility of rice bran in Leptobarbus hoevenii and Ctenopharyngodon idella. High digestibility of soybean oil cake, observed during the present study, was also evident in other fish species (Law, 1986). The digestibility of groundnut oil cake, though lower than that of soybean oil cake, was found higher when compared to the values of other plant origin feedstuffs. Oil cakes of cruciferae origin were insignificantly different in their digestibility indicating to a similarity in the

nutrient characteristics of these oil cakes. Digestibility of wheat bran showed a close proximity to that of cruciferae oil cakes. Dried silk worm pupae and slaughter house waste, with digestibility values,almost similar to fish meal, may provide a suitable alternative to the latter in formulating fish feeds. Silk worm pupae, are cheaper and available in plenty, as a byproduct of silk industry in some parts of India. Similarly, slaughter house waste, with digestibility comparable to fish meal, may provide a dependable source of cost-effective ingredient in areas where fish meal is scarce and an expensive commodity. These ingredients thus hold a strong potentiality for their use as dietary ingredients in practical rations for Indian major carps and other fishes.

The values of energy digestibility coefficient obtained for different feedstuffs in the two species of Indian major carps seemed comparable to those reported for fishes of similar feeding habit (Stickney and Lovell, 1977; Popma, 1982; NRC, 1983; Wilson and Poe, 1985; Lovell, 1989; Hossain and Jauncey, 1989).

Several factors, including size and age, are known to influence nutrient digestibility in fishes (Hastings, 1969). Ferraris et al. (1986) observed that digestibility could increase with size in omnivorous and herbivorous fishes. In the present study, however, which involved two significantly ($P < 0.01$) different size-classes of Indian major carps having distinct herbivorous/omnivorous feeding habit, no significant difference in digestibility could be seen. The close proximity of size groups used could be one of the reasons for

insignificant differences in digestibility values. In L. hoevenii, a significantly lower digestibility was demonstrated in smaller fish (5-8 cm) than the larger (12-16 cm and 20-26 cm) ones, although comparisons of digestibility between the large sized (12-16 cm and 20-26 cm) fish showed insignificant difference (Law, 1984). In Salmo gairdneri, Windell et al. (1978) reported size related differences in digestibility at low temperature only. Pandey and Singh (1980) reported that younger Colisa fasciatus (1.69-1.94 g) possess a lower capability of protein digestion than a medium sized (4.95-5.29 g) fish. However, older fish with higher weight (7.80-8.26 g) having significantly different size and weight than medium sized fish, exhibited no improvement in efficiency to digest the food protein.

A categorywise comparison of digestibility for plant and animal origin feedstuffs revealed that both the species of Indian major carps were equally effective in digesting the feedstuffs, irrespective of sources. L. rohita which predominantly feeds on plant material also consume rotifers and crustaceans (Jhingran and Pullin, 1988). C. mrigala, being omnivorous, subsists mainly on detritus which includes both plant and animal origin material. On the basis of present study, it may, therefore, be presumed that the two species are equally equipped with digestive secretions for catabolising nutrients from both the sources. Species and size-wise comparison of the ability of these fishes to digest nutrients, expressed in terms of digestibility coefficient, from the ingredients tested, point to a possible similarity in their digestive enzyme spectrum. Within the size groups investigated, in the two species

the enzyme profile does not seem to get altered with increase in fish size.

The study thus clearly demonstrates that, notwithstanding generic difference, ecological niche (column and bottom feeder), feeding habit and food composition, the two species (L. rohita and C. mrigala) do not differ much in their ability of utilizing nutrients from feedstuffs under investigation. This points to the fact that practical ration could be formulated for the polyculture of these fishes, using same feedstuffs, provided their nutrient requirement matches each other. Replacement of one feedstuff with the other, on the basis of their digestibility, cost and availability, could be attempted while formulating such diets.

SUMMARY

Apparent energy digestibility coefficient of nine different feedstuffs were determined for two different size-classes of Indian major carps, Labeo rohita (Hamilton) and Cirrhinus mrigala (Hamilton), in a flow-through system, using chromic oxide as indigestible marker. Among the ingredients tested, rice polish showed the lowest and soybean oil cake the highest digestibility in both the species. The influence of fish size, used for the study, on energy digestibility was found insignificant ($P < 0.01$). Categorywise, irrespective of fish size, plant and animal origin feedstuffs showed insignificant ($P < 0.01$) difference in their digestibility. With the

exception of animal origin feedstuffs in larger size - class, digestibility of feedstuffs, irrespective of source, were insignificant ($P < 0.01$) between the two species for both the size groups. The usefulness of the information generated has been briefly discussed.

Table 1. Details of fish size and stocking density

Species	Size groups				Stocking density (No. of fish/trough)	
	Group I		Group II			
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Group I	Group II
Labeo rohita	13.92 ±0.24	24.70 ±1.37	18.07 ±0.38	48.56 ±3.45	10	05
Cirrhinus mrigala	08.20 ±0.19	04.34 ±0.21	12.65 ±0.20	15.81 ±0.98	35	10

± SEM

Table 2. Proximate composition and gross energy content of feedstuffs (g/100 g, as fed)

Feedstuffs	Moisture	Crude Protein	Crude Fat	Crude Fibre	Ash	Gross Energy (kcal g^{-1})
Rocket salad oil cake (*Eruca sativa*)	05.89 ±0.35	40.61 ±1.46	07.98 ±0.42	09.63 ±0.28	08.01 ±0.19	04.49 ±0.04
Mustard oil cake (*Brassica campestris*)	02.64 ±0.14	37.94 ±1.17	09.82 ±0.12	09.26 ±0.20	07.79 ±0.04	04.85 ±0.03
Soybean oil cake (solv.extd.) (*Glycine max*)	07.05 ±0.46	51.05 ±2.09	00.67 ±0.16	08.52 ±0.17	07.13 ±0.12	04.55 ±0.01
Groundnut oil cake (*Arachis hypogea*)	06.34 ±0.15	47.41 ±1.15	05.76 ±0.95	08.29 ±0.27	06.75 ±0.43	04.65 ±0.01
Wheat bran (*Triticum aestivum*)	10.86 ±0.71	15.82 ±0.41	04.89 ±0.10	10.26 ±0.20	06.19 ±0.08	04.51 ±0.04
Rice polish (*Oryza sativa*)	09.06 ±0.14	09.90 ±0.49	11.57 ±0.52	03.20 ±0.31	05.18 ±0.17	04.57 ±0.01
Fish meal (mixed spp.)	06.52 ±0.30	59.78 ±0.39	11.48 ±0.31	01.00 ±0.10	19.87 ±0.21	04.64 ±0.07
Slaughter house waste	04.78 ±0.05	77.98 ±0.82	10.24 ±0.17	00.95 ±0.09	03.47 ±0.01	05.36 ±0.09
Dried silkworm pupae (*Bombyx mori*)	02.52 ±0.18	40.69 ±1.24	21.73 ±0.29	13.23 ±0.20	04.20 ±0.12	05.67 ±0.04

± SEM

Table 3. Ingredient composition of experimental diets

Feedstuffs (Main ingredients)	Percentage composition, as fed			
	Main ingredient	Crude Fibre[1]	Oil[2]	Others[3]
Rocket salad oil cake	87.50	06.0	1.00	5.5
Mustard oil cake	86.50	07.0	1.00	5.5
Soybean oil cake (solv.extd.)	79.50	07.0	8.00	5.5
Groundnut oil cake	83.50	08.0	3.00	5.5
Wheat bran	84.50	06.0	4.00	5.5
Rice polish	80.50	13.0	1.00	5.5
Fish meal (mixed spp.)	79.17	15.0	0.33	5.5
Slaughter house waste	78.17	16.0	0.33	5.5
Dried silkworm pupae	91.17	03.0	0.33	5.5

[1] 1:1 α-cellulose and sugarcane bagasse

[2] 2:1 corn and cod liver oil

[3] Carboxymethyl cellulose 2.0%; gelatin 1.0%; Vit. mix. 1.0%; mineral mix. 1.0%; and chromic oxide 0.5%

Table 4. Energy digestibility coefficient of selected feedstuffs in different size-classes of Indian major carps

Feedstuffs	Coefficient of energy digestibility (%)			
	L. rohita		*C. mrigala*	
	Group I	Group II	Group I	Group II
Rocket salad oil cake	63.32^{ab} ±0.88	76.04^{a} ±0.29	63.07^{a} ±0.44	74.77^{a} ±1.25
Mustard oil cake	64.33^{ab} ±1.09	63.14^{bc} ±2.82	50.27^{bc} ±1.99	55.75^{bc} ±3.37
Soybean oil cake (solv.extd.)	96.18^{d} ±1.28	90.00^{d} ±0.40	81.27^{d} ±0.24	89.83^{d} ±1.11
Groundnut oil cake	78.36^{e} ±1.48	82.98^{e} ±0.29	76.82^{e} ±0.74	81.97^{e} ±0.96
Wheat bran	58.58^{b} ±0.60	63.02^{c} ±1.51	44.69^{c} ±0.01	53.86^{bc} ±4.15
Rice polish	48.80^{f} ±3.52	43.55^{f} ±1.72	45.82^{c} ±5.24	49.07^{c} ±2.97
Fish meal (mixed spp.)	69.34^{a} ±0.62	72.64^{ab} ±0.70	53.97^{abc} ±3.27	58.03^{bc} ±6.48
Slaughter house waste	61.23^{ab} ±2.82	69.51^{abc} ±6.47	57.41^{ab} ±1.78	64.09^{b} ±2.89
Dried silkworm pupae	66.46^{ab} ±2.47	68.01^{abc} ±1.45	58.34^{ab} ±2.01	60.27^{bc} ±6.08

± SEM

Means in each column with same superscript are insignificantly different ($P<0.01$)

CHAPTER 3

INFLUENCE OF FEEDING VARYING LEVELS OF DIETARY ENERGY ON THE GROWTH, UTILIZATION EFFICIENCY AND CARCASS COMPOSITION OF THE INDIAN MAJOR CARP, CIRRHINUS MRIGALA (HAMILTON) FRY

INTRODUCTION

Although notable success has been achieved in semi-intensive culture of Indian major carps through indigenous development and standardization of various artifacts of aquaculture, lack of proper feed development, requiring a precise knowledge of nutritional needs of the concerned species, remains a major impediment towards more intensive culture of these fishes. As already stated, nutritional studies on Indian major carps had been confined mainly to quantifying their protein and amino acid requirements (Sen et al., 1978; Singh and Bhanot, 1988; De Silva, and Gunasekera, 1991; Khan, 1991; and Ravi and Devaraj,1991), with almost no information on other aspects of nutrition.

The Indian major carp, Cirrhinus mrigala, is a fast growing fish, attaining marketable size of 800-1000 g in less than a year (Jhingran and Pullin, 1988). It is used as a component of polyculture with other species of major carps. The protein requirement of C. mrigala is reported to be between 40-50% of the diet (Khan, 1991 and De Silva and Gunasekera, 1991). In fishes, besides other factors, the source and level of dietary energy are

known to influence the utilization of dietary protein vis-a-vis growth (Steffens,1981). Dietary energy density also influences the carcass composition of fish (Zeitler et al., 1984). The present study was aimed to examine the influence of feeding varying levels of dietary energy density on the growth, utilization efficiency and carcass composition of C. mrigala fry.

MATERIALS AND METHODS

Preparation of experimental diets

Six semipurified, isonitrogenous diets with varying levels of metabolizable energy (280.90, 310.06, 339.81, 367.00, 381.65, 396.31 kcal/100 g, as fed) were formulated (able 1). Physiological fuel values used for dietary energy calculation were the same as given elsewhere (page 23). Details of preparation of diets are given under General Methodology section (page 13). To obtain the required energy levels, the amounts of dextrin and lipid were gradually increased in the diet at the expense of α -cellulose. The energy to protein ratio (kcal g^{-1}) in the diets ranged between 7.02-9.90.

Feeding trial

Source of fish, details of their acclimation and general experimental design are given under General Methodology section (page 12-14). Triplicate groups of 35 fish each (2.0±0.3 cm and 0.40±0.02 g) were stocked in experimental set up similar to that

used for the acclimation of fish.

Initially, feeding rate was fixed at 8% (calculated as dry feed/wet fish weight) of body weight day^{-1}. It was noted that the fish readily consumed the daily ration, therefore, ration level was raised from second week onwards to 10% of the body weight day^{-1}. Water temperature and dissolved oxygen, over the 4-week experimental period, were 27±2°C and 6.2± ppm, respectively.

Proximate analyses

A group of 20 fish was randomly taken out from the acclimated fish lot for the analyses of initial carcass composition using the standard technique as detailed on page 14-17. At the end of the feeding trial, 15 specimens from each trough were again taken randomly, pooled and analysed similarly for their final carcass composition.

Statistical analyses

Data on weight gain (%), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV) were subjected to one-way analysis of variance. Comparison between treatment means were made using Duncan's new multiple range test (Duncan, 1955). Second order polynomial regression analysis (Snedecor and Cochran, 1967) was employed to weight gain (%) data to predict maximum gain in response to energy level (Fig. 1).

Significantly correlated carcass components were fitted to regression equations. Test of significance of the regression equations were performed using Student's t-test.

RESULTS

Over the 4-week feeding trial, the average survival in each treatment was over 90%. No specific pattern of mortality was noticed to which treatment levels could be related (Table 2).

Increasing dietary energy produced significantly different ($P < 0.01$) weight gains (%), over the experimental period, following a quadratic pattern (Table 2). Diets containing low (280.9-310.06 kcal/100 g) energy produced significantly ($P < 0.05$) lower weight gains. Weight gain increased with energy level, reaching the maximum at 367.00 kcal/100 g, beyond which growth was found to depress. The relationship between weight gain and energy level, depicted through a polynomial curve (Fig. 1), indicate that, with the form of energy provided, maximum gain could be achieved at 355.00 kcal/100 g.

The mean FCR, PER and PPV of each fish group are given in Table 2. These values differed significantly ($P < 0.01$) among the various treatment levels. FCR, PER and PPV improved with dietary energy, registering their maximum at 381.65 kcal/100 g. Fish group fed diet with an energy level of 339.81 kcal/100 g produced statistically comparable result with respect to above parameters.

Increasing energy to 396.31 kcal/100 g showed no further improvement in conversion efficiencies.

The body composition of fish fed the test diets is given in Table 3. Body moisture, ash, crude protein and fat were significantly ($P < 0.01$) affected by dietary treatments. At the end of the study, compared to the initial values, all the experimental groups exhibited a higher percentage of fat and lower percentages of moisture and ash. Body moisture was inversely ($Y = 82.390 - 0.8318\ X$; $n = 18$; $r = -0.741$; $P < 0.05$) related to dietary energy levels. Both protein and ash were negatively correlated to dietary energy, and the equations describing the relationship of percent crude protein ($Y = 104.560 - 4.1834\ X$; $n = 18$; $r = -0.95$) and ash ($Y = 20.05 - 1.1721\ X$; $n = 18$; $r = -0.96$) with calorie content of the diet were significant ($P < 0.05$). However, when carcass protein was calculated on fat free basis, no such relationship could be found. Carcass fat was directly ($Y = 23.93 + 4.929\ X$; $n = 18$; $r = 0.95$; $P < 0.05$) related to dietary energy levels. Carcass protein and ash content showed inverse ($r = -0.99$) relationship with body fat.

DISCUSSION

The differential growth obtained in C. mrigala during the present study seems related to variations in dietary calorie density. The quadratic growth pattern noted for this fish was found similar to that reported for several other fish species (Ringrose, 1971;

Takeuchi et al., 1978[c]; Millikin, 1983; Henken, et al., 1986; Tabachek, 1986; Barrows, et al., 1988), with the exception of Sciaenops ocellatus (Daniels and Robinson, 1986), Sparus aurata (Marais and Kissil, 1979) and Siganus guttatus (Parazo, 1990), where decreased or increased growth occurred with increasing levels of dietary energy.

With dietary energy being the primary source of variation in growth rate, the lower weight gain noticed at low energy diet could be the result of insufficient energy consumption. Fixed ration (10%) level might also have restricted the fish from consuming more feed to compensate insufficient energy supply from low energy diets. As a result, the fish presumably catabolised dietary protein to meet its requirements for energy than growth. Gradual increase in growth performance, with each incremental level of dietary energy, up to 367.00 kcal/100 g strengthens the fact that with increase in energy availability more protein was utilized for tissue building and hence growth enhancement. This assumption may gain further support from the increased efficiency of protein, expressed in terms of PER and PPV, and betterment of FCR observed with increasing dietary energy intake.

It has been established that fish eat to satisfy their energy requirement, and several earlier workers (Lee and Putnam, 1973; and Page and Andrews, 1973) have attributed low growth rate at high energy diet to reduced consumption of feed, depriving fish from essential nutrients. The decrease in growth rate noted with

dietary energy level exceeding 367.00 kcal/100 g, could thus be related to the fact that C. mrigala consumed their daily requirement of energy before necessary protein or other nutrient requirements were met. Reduction in growth rate in fishes fed high energy diet were also reported in channel catfish (Page and Andrews, 1973; Lovell, 1979), rainbow trout (Beamish and Medland, 1986) and red tilapia, (De Silva et al., 1991).

Growth rate and feed conversion, which reflect the performance of a farming system, are greatly influenced by the nutrient composition of feed, including its energy density. Better feed conversion in C. mrigala with increased dietary energy indicates its beneficial effect on feed utilization. Several factors, including dietary energy content and source, influence protein utilization (Steffens, 1981). Influence of energy density and non-protein energy sources on the efficiency of utilization of contained protein (40%) in the present experiment on C. mrigala can be assessed by comparing the values of PER and PPV of different diets. The increased protein utilization, measured in terms of PER and PPV, with increasing dietary energy level suggests towards greater use of dietary protein for fish growth up to 381.65 kcal/100 g energy incorporation.

In the present study, the level (7-12%) of lipid incorporation in the experimental diets were kept slightly higher than the reported requirement for C. mrigala (Anonymous, 1991) so as

to ensure sufficient supply of both ω_3 and ω_6 fatty acids to the fish. The percent calorie contribution of carbohydrate, as non-protein calorie source in the diet, was in increasing order, up to 367.00 kcal/100 g where maximum weight gain was noticed, whereas contribution from lipid was in the reverse order. This indicates that it was carbohydrate which helped greater utilization of dietary protein towards growth. The fact is strengthened by the pattern of changes observed in PER and PPV values. Anderson et al. (1984) reported protein sparing effect of dietary carbohydrate in omnivorous tilapia fed graded levels of carbohydrates in isonitrogenous (35% C.P.) diets. Sparing of protein for growth by dietary carbohydrate was also demonstrated in omnivorous channel catfish (Garling and Wilson, 1977). Carnivorous fishes like salmonids (Pieper and Pfeiffer, 1979; and Bergot, 1979), plaice (Cowey et al., 1975), turbot (Adron et al., 1976) and eels (Degani, 1987) also use carbohydrate as an energy source, sparing protein for growth.

Growth depression and somewhat poor conversion efficiency values beyond 381.65 kcal/100 g noticed in C. mrigala may not be related to high carbohydrate (32.66%) incorporation which represented approximately 29% of the total energy in the diet. Anderson et al. (1984) observed no growth depressing effect in tilapia with diet containing 40% carbohydrate (representing approx. 30% of gross dietary energy). However, Fruichi and Yone (1980) have shown growth retardation in carp receiving 40% carbohydrate

diet. The highest weight gain obtained in C. mrigala fed diet with a maximum of 34% carbohydrate indicates that this level of dietary carbohydrate inclusion is within the tolerance limit of the fish. Since diet containing 28.66% carbohydrate has resulted in (almost) similar performance, in terms of weight gain, FCR, PER and PPV, as diet having 34% carbohydrate, the former level of carbohydrate inclusion may be suggested for practical feed development. This favourably compares with the requirement values reported for carps (Sen et al., 1978) and tilapia (Anderson et al., 1984). Although common carp, Cyprinus carpio, is reported to be efficient in utilizing very high level of dietary lipid (Jauncey, 1982[b]), the growth depressing effect noted in C. mrigala due to higher level (12%) of dietary lipid inclusion, adding to increased energy content in the diet, cannot be ruled out.

Diet has often been shown to have profound influence on body composition of fish (Brett et al., 1969; Buckley and Groves, 1979; Huisman et al., 1979). Among the various constituents, carcass fat has been reported to show the greatest fluctuation in carp (Zeitler et al., 1984). The positive correlation noted between dietary energy level and carcass fat content in C. mrigala compare favourably with the results obtained on channel catfish (Page and Andrews, 1973), African catfish (Henken et al., 1986) and tilapia (Shiau and Huang, 1990; De Silva et al., 1991). Anderson et al. (1984) correlated dietary energy density, contributed by carbohydrate, with

carcass fat in tilapia.

In C. mrigala, increase in dietary energy level, either with carbohydrate (dextrin) or fat, resulted in concomitant increase in carcass fat. Efficient dietary carbohydrate utilization and higher deposition of fat in the carcass of this fish seem comparable to the observation of Likimani and Wilson (1982) on channel catfish, fed high carbohydrate diets, where increased lipid deposition in carcass was attributed to lypogenic enzyme activity.

The inverse relationship between dietary calorie density and carcass protein or ash content in C. mrigala conforms with similar observations on several other fish species (Page and Andrews, 1973; Zeitler et al., 1984; and De Silva et al., 1991) but appears contradictory to the findings on tilapia (El-Sayed and Teshima, 1992). It may be stressed here that when carcass protein in C. mrigala was calculated on fat free basis, no notable difference occurred among the fish groups receiving various experimental diets. A corollary to this observation was apparent in the work of Page and Andrews (1973), Buckley and Groves (1979) and Parazo (1990). The negative correlation between dietary energy levels and carcass protein or ash content may be linked to the diluting effect of carcass fat. This becomes clear from the inverse relationship found between carcass fat and protein/ash. Sabaut and Luquet (1973), and Marais and Kissil (1979) have, however, observed no statistical difference in the body composition of fish fed diets containing different energy levels.

When relationship between dietary energy level and growth rate was plotted for C. mrigala, the maximum weight gain was obtained at an energy density of 355 kcal/100 g. Duncan's multiple range analysis of the data on the performance of fish also suggested a calorie level between 339.81 and 367.00 kcal/100 g, as optimum for desirable performance of diet.

The dietary E/P ratio of 8.87, corresponding to the energy level of 355 kcal/100 g, obtained through polynomial curve (Fig.1) in C. mrigala, compare favourably with the value reported for C. carpio (Takeuchi et al., 1979). Stickney and Lovell (1977) recommended an E/P ratio in the range of 8 to 9 for fingerling and production diets of channel catfish, stating that the same range may be applicable to other warmwater fish species. On the basis of present study on C. mrigala, it may conclusively be stated that a minimum of 355.00 kcal/100 g is essential for efficient nutrient utilization and growth of this detritivorous carp species, and that practical diets can be formulated for this species with E/P ratio of 8.87 in a 40% CP diets, incorporating 29.5% of metabolizable energy as carbohydrate.

SUMMARY

Six semipurified isonitrogenous (40% C.P.) diets containing varying levels of metabolizable energy (280.90, 310.06, 339.81, 367.00, 381.65, and 396.31 kcal/100 g, as fed) were fed to Cirrhinus mrigala fry in 70-1 troughs fitted with flow-through system for

4-weeks. Over the experimental period, weight gain was significantly ($P < 0.01$) affected by dietary energy levels following a quadratic pattern. A second degree polynomial curve indicates that an energy level of 355.00 kcal/100 g, corresponding to an E/P ratio of 8.87 (kcal g^{-1}), would produce the maximum weight gain. Efficiency of feed and protein conversion, in terms of FCR, PER, and PPV, increased up to certain level of energy incorporation. Carcass composition was significantly ($P < 0.01$) affected by dietary energy. The relationship between energy levels and various carcass components was discussed. The significance of the above findings has been briefly elucidated.

Table 1. Ingredient and proximate composition of test diets

Ingredients (g/100 g, as fed)	Diets					
	I	II	III	IV	V	VI
Casein (Vitamin free, 84% C.P.) [1]	35.71	35.71	35.71	35.71	35.71	35.71
Gelatin (87.6% C.P.)[2]	11.41	11.41	11.41	11.41	11.41	11.41
Dextrin	11.83	20.16	28.66	34.00	33.33	32.66
Corn oil	04.67	04.67	04.67	05.34	06.67	08.00
Cod liver oil	02.33	02.33	02.33	02.66	03.33	04.00
Mineral mix.	04.00	04.00	04.00	04.00	04.00	04.00
Vitamin mix.	01.00	01.00	01.00	01.00	01.00	01.00
∝-cellulose	28.05	19.72	11.22	04.88	03.55	02.22
Carboxymethyl cellulose	01.00	01.00	01.00	01.00	01.00	01.00
Proximate composition % (calculated)						
Crude protein	40.00	40.00	40.00	40.00	40.00	40.00
Crude fat	07.00	07.00	07.00	08.00	10.00	12.00
Carbohydrate	11.83	20.16	28.66	34.00	33.33	32.66
Metabolizable energy (kcal/100 g, as fed)	280.90	310.06	339.81	367.00	381.65	396.31
Carbohydrate calories (%)	14.74	22.75	29.51	32.42	31.26	28.84
Lipid calories (%)	21.18	19.18	17.50	18.52	20.50	25.73
Energy/protein ratio (kcal/g protein)	07.02	07.75	08.49	09.17	09.54	09.90

[1] ICN Pharmaceuticals, Cleveland, Ohio, USA; [2] Loba Chemi, India.

Table 2. Results of feeding experimental diets to C. mrigala fry during a 4-week growth trial

	Diets					
	I	II	III	IV	V	VI
Initial individual wet weight (g)	00.39 ±0.002	00.41 ±0.007	00.42 ±0.001	00.43 ±0.001	00.42 ±0.004	00.37 ±0.019
Final individual wet weight (g)	00.77 ±0.100	00.91 ±0.008	01.00 ±0.009	01.08 ±0.011	01.04 ±0.008	00.84 ±0.036
Percent increase in weight	95.02^{a} ±1.50	119.92^{b} ±6.40	134.51^{ce} ±2.66	148.47^{d} ±1.25	147.05^{cd} ±0.74	124.34^{be} ±2.10
Feed conversion	03.16^{a} ±0.05	02.60^{b} ±0.10	02.35^{c} ±0.03	02.20^{ce} ±0.04	01.98^{d} ±0.006	02.03^{de} ±0.02
Protein efficiency ratio	00.78^{a} ±0.008	00.96^{b} ±0.03	01.06^{bc} ±0.01	01.11^{bcd} ±0.01	01.26^{d} ±0.02	01.22^{cd} ±0.01
Protein productive value	19.15^{a} ±0.17	21.61^{b} ±0.57	23.13^{c} ±0.21	24.05^{ce} ±0.28	26.09^{d} ±0.35	25.43^{de} ±0.13
Percent survival	91	96	94	100	97	94

Results are means ± SE of triplicate fish groups. Values in each row with same superscript are insignificantly different ($P<0.01$)

Table 3. Carcass composition of C. mrigala fry fed the experimental diets

g/100 g, dry matter	Initial	Diets					
		I	II	III	IV	V	VI
Moisture*	82.97 ±0.16	76.25^{a} ±0.55	76.13^{ad} ±0.88	75.53^{ad} ±0.12	76.02^{ad} ±0.17	74.03^{bd} ±0.52	73.42^{c} ±0.50
Crude protein	65.39 ±1.39	73.91^{a} ±1.32	72.59^{ab} ±0.89	70.59^{ab} ±1.01	67.82^{b} ±0.77	64.47^{c} ±1.21	61.91^{d} ±1.11
Crude fat	08.52 ±0.70	12.09^{a} ±0.90	13.89^{ac} ±0.14	16.15^{bc} ±0.14	19.27^{b} ±0.00	23.24^{d} ±1.24	26.39^{d} ±0.33
Ash	23.15 ±1.48	11.48^{a} ±0.27	11.10^{a} ±0.02	10.49^{a} ±0.02	09.74^{b} ±0.28	08.89^{c} ±0.21	08.09^{d} ±0.24

*g/100 g, wet weight

Results are means ± SE of triplicate fish groups. Values in each row with same superscript are insignificantly different ($P < 0.01$)

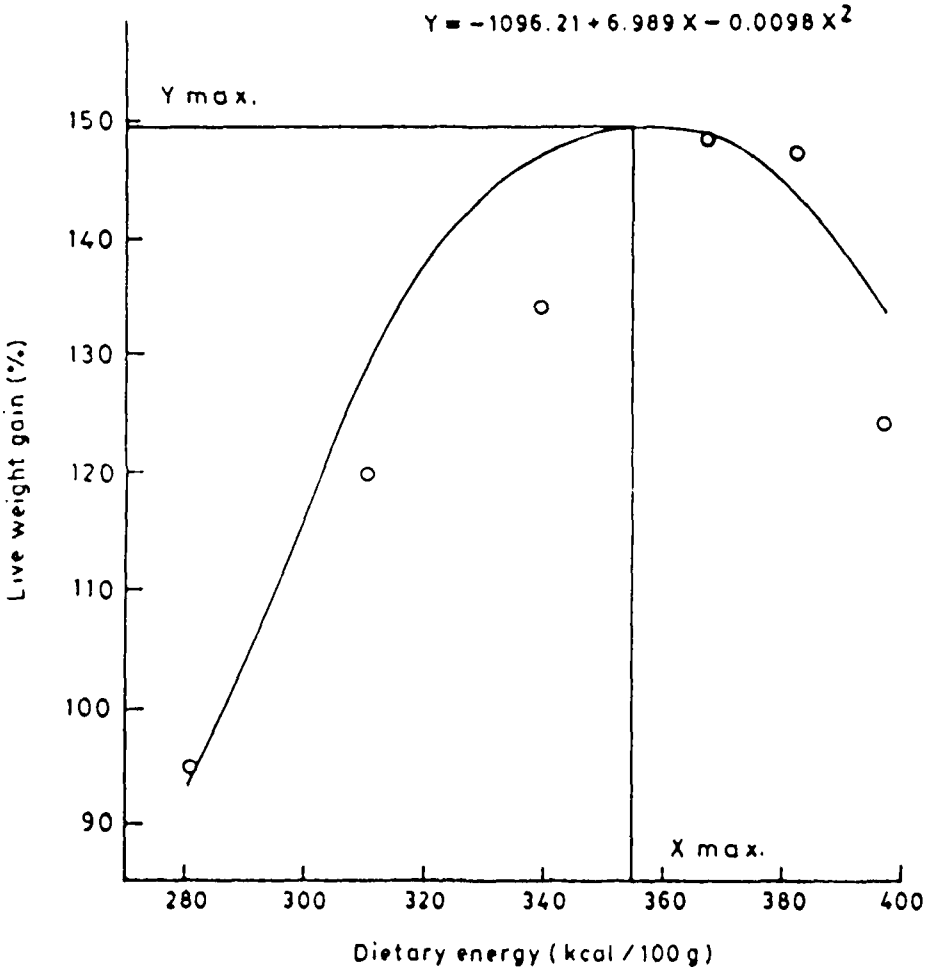

Fig. 1. The second degree polynomial relation of percent live weight gain and dietary calorie density in C. mrigala.

CHAPTER 4

DIETARY ENERGY AND PROTEIN INTERACTION - AN APPROACH TO OPTIMIZING ENERGY : PROTEIN RATIO IN INDIAN MAJOR CARP, CIRRHINUS MRIGALA (HAMILTON) FINGERLING

INTRODUCTION

Fish require relatively high amount of protein in their diet which, besides contributing to higher cost of feeding, deteriorates water quality through excessive excretion of nitrogenous (NH_3) wastes. To make aquaculture economically more viable and environmentally sustainable, studies are warranted with the aim to reducing protein component in the diet without compromising the growth or utilization efficiencies in fish. Earlier attempts to reduce the amount of protein in fish diet were confined mainly to fishes like salmon, trout and channel catfish (Lee and Putnam, 1973; Page and Andrews, 1973; Garling and Wilson, 1976; Reinitz et al., 1978; Takeuchi et al., 1978[ab]). It has been pointed out that provision of sufficient amount of nonprotein energy in the diet, to meet the daily needs and metabolic activity of the body, results in greater utilization of protein for growth (Wilson, 1989). The sparing action of nonprotein energy, particularly from lipid, has been reported for several temperate fish species but information on tropical fishes are limited (Winfree and Stickney, 1981; Shiau and Huang, 1990; De Silva et al., 1991 and El-Sayed and Teshima, 1992), and similar studies on Indian major carps are lacking. An attempt has, therefore,

been made to investigate the dietary energy-protein interaction, leading to the optimization of energy to protein ratio in Cirrhinus mrigala fingerling, and the findings are reported in this chapter. The data will be useful in formulating cost-effective diet for this fish.

MATERIALS AND METHODS

Preparation of experimental diets

Casein-gelatin based semipurified diets were formulated (Table 1) to contain 30 and 40% crude protein, and each tested at three different levels (average 313.56±4.85; 360.77±4.94; 407.95±5.79 kcal/100 g) of energy in a 2 X 3 factorial design. Method of preparation of the diet is given on page 13. Physiological energy values adopted for dietary energy calculation were same as mentioned in Chapter 1. To obtain the required energy levels, the amount of lipid was gradually increased in the diet at the expense of dextrin and α-cellulose. The energy to protein ratio E/P in the experimental diets ranged between 7.75-13.73 kcal/g protein.

Feeding trial

Fingerling (4.3-7.1 cm; 3.67-3.94 g) of C. mrigala were netted out from stocking pond (see page 12) and stocked in polyvinyl circular troughs (water volume and exchange rate being 55 l and 1 l/min, respectively) for a 3-week acclimation to experimental diets.

For each dietary treatment, triplicate groups of 10 fish were randomly assigned to one of the six experimental diets and the trial continued for 7 weeks. Variations in initial body weight, between the treatment groups, were minimized, as far as possible, to avoid any possible effect on final weight gain. Fish were fed twice daily at the total rate of 5% of body weight at 0800 and 1600 h. After a stipulated period of feeding, unconsumed feed, if any, was collected on a fine mesh sieve and dried (100°C). Other details of the general experimental design were the same as given elsewhere (page 13-14). Water temperature over the experimental period was 25±1°C.

Growth parameters and utilization efficiencies were measured using standard definitions (page 19-21).

Gross energy and proximate analyses

Initial proximate carcass analyses and gross energy estimates were made on fish, randomly taken out from the acclimated lot, using standard techniques (page 14-18). On the termination of feeding trial, required number of fish from each trough were again taken out and analysed similarly for gross energy and final carcass composition.

Statistical analysis

To test the effect of dietary energy and protein, and their interaction, on fish growth, feed utilization, gross energy and carcass

composition, the data were subjected to two-way analysis of variance using the model :

$$Y_{ijk} = \mu + P_i + l_j + pl_{ij} + e_{ijk},$$

where,

Y_{ijk} = measurement of weight gain, feed utilization or whole body composition,

μ = the overall mean,

P_i = the effect of ith level of dietary protein,

l_j = the effect of jth level of dietary energy,

pl_{ij} = the interaction effect for the ith level of dietary protein and jth level of dietary energy, and

e_{ijk} = the random error associated with individual observations for ith level of dietary protein, jth level of dietary energy and kth number of replicates.

When significant differences were detected, with no significant interaction between dietary energy by ANOVA, the treatment means were compared using Duncan's new multiple range test (Duncan, 1955). On the other hand, when a significant interaction appeared, the various treatment means were compared only at either of the iso-nitrogenous treatment groups.

Relationship between specific growth rate (SGR) and dietary energy level was established using second degree polynomial analysis

(Snedecor and Cochran, 1967). Relationship between dietary energy level and different carcass components were worked out through regression analysis. Significantly correlated body constituents were fitted to regression equation.

RESULTS

In the present study, two-way ANOVA revealed that weight gain, feed conversion (FCR) and protein efficiency (PER) ratios (Table 2 and 4) in fingerling C. mrigala were significantly influenced both by dietary energy and protein levels. However, interactive effect of dietary energy and protein was not evident on the growth performance of fish. Daily average growth increment, as measured by specific growth rate, indicated a quadratic pattern in relation to dietary energy at both the protein levels tested (Fig.1). After the maximum gain at an average of 360.77 kcal/100 g, a fall in the rate of growth was noticeable with increase in dietary energy intake. However, when isocaloric diets were compared, betterment of growth performance was seen with increased protein levels.

Increasing energy with either of the protein levels, up to an average of 360.77 kcal/100 g, improved feed conversion. FCR within isocaloric diets were better in fish groups receiving 40% C.P. diet. PER indicated a similar pattern of changes in relation to energy content. It decreased with dietary protein, when isocaloric diets were compared. Energy conversion efficiency (ECE) and protein

productive value (PPV) were significantly influenced both by dietary energy and protein, and their interactive effect (Table 2 and 4). Although PPV was inversely related to protein, when isocaloric diets were compared, it showed insignificant difference up to 360.77 kcal/100 g, energy inclusion, at both the protein level. However, ECE was found directly related to protein levels in the diet and it levelled off beyond an average of 360.77 kcal/100 g energy incorporation.

Proximate analyses of whole body composition of fish have been summarized in Table 3. Interactive effects of dietary energy and protein levels were not evident on any of the body constituents, excepting moisture. Body constituents were found significantly affected by dietary energy but influence of dietary protein was not seen. Compared to initial values, the various experimental groups exhibited lower percentages of ash, protein and moisture and higher percentage of fat at the end of the experiment.

At both the protein levels, the whole body fat (Y) content was positively correlated (30% C.P. : $r = 0.914$, $P < 0.1$; 40% C.P.: $r = 0.879$, $P < 0.1$) with dietary energy (X), the relationship being expressed by the equations $Y = -21.591 + 13.02\ X$ for 30% C.P., and, $Y = -24.734 + 14.23\ X$ for 40% C.P. diets. However, body crude protein (Y) correlated negatively (30% C.P. : $r = -0.987$, $P < 0.05$; 40% C.P. : $r = -0.99$, $P < 0.05$) with dietary energy (X), irrespective of protein level in the diet. This relationship can be understood from the equation $Y = 125.583 - 18.498\ X$ for 30%

C.P., and Y = 122.4 - 18.138 X for 40% C.P. diets.

Coefficient of correlation between whole body fat (X) and protein (Y), at either of the protein levels, indicated a significant negative relationship (30% C.P. : $r = -0.96$, $P < 0.1$; 40% C.P. : $r = -0.98$, $P < 0.1$), obtaining the equation Y = 91.077 - 1.272 X for 30% C.P., and Y = 89.348 - 1.215 X for 40% C.P. diets. The whole body moisture followed the pattern of changes (30% C.P. : $r = -0.99$, $P < 0.05$; 40% C.P. : $r = -0.94$, $P < 0.05$), similar to that of body protein, in relation to body fat. The equations describing the relationship between body moisture and fat (X) were Y = 82.849 - 0.33 X, and Y = 78.71 - 0.156 X at 30% and 40% C.P. diets, respectively. An inverse relationship also existed between body fat and ash.

DISCUSSION

The quadratic growth pattern observed in fingerling C. mrigala conforms with the pattern noted for the fry of this species (Chapter 3). Although quadratic effect of energy on weight gains, within the isonitrogenous diets, has been reported in other fish species (Watanabe et al., 1979; Millikin, 1983; Henken et al., 1986; Barrows et al., 1988; and De Silva et al., 1991), a linear increase (El-Sayed and Teshima, 1992) or decrease (Daniels and Robinson, 1986) has been pointed out in Nile tilapia and red drum, respectively. Maximum growth of C. mrigala noted at 40% dietary crude protein coincide with the protein requirement value reported

by Khan (1991), through a dose-response study, for the fingerling of this species. Increase in the growth with dietary protein, within isocaloric diets, suggested an E/P ratio of 8.93 for optimum growth of the fish. Almost similar value of E/P ratio (8.87), obtained from a polynomial curve, was found optimum for C. mrigala (Chapter 3). Significant influence of dietary energy and protein on percent weight gain revealed growth accelerating effect of energy intake only up to a certain level with proper combination of protein intake (E/P ratio).

Data on FCR also clearly indicate that both energy and protein components of diet influence feed conversion in fingerling C. mrigala, pointing towards a beneficial effect of sufficient energy incorporation in diet. Increase in feed efficiency and weight gain up to certain level of energy inclusion (through lipid) has been reported by earlier workers as well (Page and Andrews, 1973; Takeuchi et al., 1978[b]; Dupree et al., 1979; Watanabe et al., 1979; Shiau and Huang, 1990, and De Silva et al., 1991). A fall in growth rate and feed conversion of C. mrigala fed very high energy diet could be attributed either to decreased feed intake, due to its high energy content (Page and Andrews, 1973), or to the hindrance of digestion and absorption of other nutrients by high fat content in the diet (Dupree et al., 1979).

Significant influence of both dietary energy and protein on PER and PPV demonstrate the importance of these variables on protein utilization. Although the pattern of changes in PER in relation to dietary energy show similarity with

the observations of De Silva et al. (1991) on red tilapia, and that of El-Sayed and Teshima (1992) on Nile tilapia, it contradicts the findings of Reinitz et al. (1978), Watanabe et al. (1979), Tabachek (1986), and Shiau and Huang (1990) who noted a positive linear relationship between dietary energy (from lipid) and protein utilization. Better PER with decreasing levels of dietary protein, as observed in C. mrigala, has also been reported for other fishes (Millikin, 1983; Daniels and Robinson, 1986; Parazo, 1990). Similarly, the inverse correlation between PPV and dietary protein level finds corollary in the work of Millikin (1983) on striped bass. For better protein conversion, incorporation of energy in the diet beyond an average of 360.77 kcal/100 g proved wasteful, in terms of PPV, in C. mrigala. Calorie contribution from lipid up to 35% to the total average dietary calorie of 360.77 kcal/100 g revealed a beneficial effect of dietary lipid, both in terms of protein utilization as welll as protein retention, in C. mrigala. A judicious reduction of protein to the extent of 30% could be made, while formulating practical diet, if protein conversion is considered as the basic purpose of fish culture, to economise the utilization of costly protein. De Silva et al. (1991) noted protein sparing effect up to 18% lipid inclusion in tilapia.

In C. mrigala fingerling, dietary energy, protein, and their interaction, seemed to influence energy conversion. At both the protein levels, energy conversion plateaued beyond 360.77 kcal/ g energy incorporation in the diet, and this may be considered as requirement level of energy, since surplus energy neither helped protein

utilization nor growth, it rather got deposited in the form of body lipid. This indicates that at higher dietary protein level some amount of protein also contributes to energy retention. This assumption finds support in the work of Lee and Putnam (1973) and Cowey (1979), who maintained that in fish fed at excessive protein and energy levels excess protein is used as energy, leading to fat deposition thereby adding to calorific value of flesh. However, high calorie content in early stages may prove advantageous to fish, if released in the natural environment for stock replenishment.

Influence of dietary protein on body constituents of fish was insignificant over the period of feeding trial. The observation seems in agreement with the findings of Page and Andrews (1973), Reinitz et al. (1978), Millikin (1982), Daniels and Robinson (1986), and De Silva et al. (1991), but differs from that of Lee and Putnam (1973), Austreng and Refstie (1979), and Millikin (1983) who observed increase in whole body protein with dietary protein on feeding fish for ten weeks or longer. A positive correlation of dietary energy with body lipid and a negative relationship with body protein, noted in C. mrigala, conform with similar finding on other fishes. Watanabe (1982) has stated that whole body lipid in fish generally increases with dietary lipid. The fact that inverse relationship of body protein with dietary energy is not because of decreased absolute level of protein, but due to dilution by body fat, finds strength by the negative correlation observed in C. mrigala between body fat and protein components. Decrease in body moisture and ash with increasing dietary energy, at both the protein

levels, conform with the results on tilapia (Winfree and Stickney, 1981, and Shiau and Huang, 1990), channel catfish (Garling and Wilson, 1976), and carp (Zeitler et al., 1984).

SUMMARY

In a 7-week feeding trial, conducted in 70 l flow through type polyvinyl circular troughs at 25±1°C, two levels of dietary protein (30 and 40%) having three energy levels (average 313.56±4.85, 360.77±4.77 and 407.95±5.79 kcal/100 g) each, were used to determine the optimum dietary energy to protein ratio in fingerling C. mrigala. Factorial analysis of variance indicated that although weight gain, feed and protein utilization efficiencies were significantly affected by dietary energy and protein levels, the energy and protein conversion efficiencies were influenced both by the levels of energy and protein in diet, and their interaction. Best growth and feed conversion were obtained with an average of 360.77 kcal/100 g at 40% C.P. diet (E/P, 8.93) while maximum protein utilization and conversion occurred with similar energy but low (30%) protein in diet (E/P, 12.13). Although significant influence of dietary energy was evident on all the body components of fish, the influence of protein was insignificant. Interrelationships of various body constituents were established and discussed.

Table 1. Ingredient and proximate composition of experimental diets

Ingredients (g/100 g, as fed)	**Diets**					
	I	**II**	**III**	**IV**	**V**	**VI**
Casein (Vitamin free; 84% C.P.)[1]	26.78	26.78	26.78	35.71	35.71	35.71
Gelatin (87.6% C.P.)[2]	08.56	08.56	08.56	11.41	11.41	11.41
Dextrin	35.00	29.00	23.30	20.18	14.30	08.10
Corn oil	04.67	10.00	15.34	04.67	10.00	15.34
Cod liver oil	02.33	05.00	07.66	02.33	05.00	07.66
Mineral mix.	04.00	04.00	04.00	04.00	04.00	04.00
Vitamin mix.	01.00	01.00	01.00	01.00	01.00	01.00
∝-cellulose	14.66	12.66	10.36	17.70	15.58	13.78
Carboxymethyl cellulose	03.00	03.00	03.00	03.00	03.00	03.00
Proximate composition % (calculated)						
Crude protein	30.00	30.00	30.00	40.00	40.00	40.00
Crude fat	07.00	15.00	23.00	07.00	15.00	23.00
Carbohydrate	35.00	29.00	23.00	20.00	14.00	08.00
Metabolizable energy (kcal/100 g, as fed)	317.00	364.00	412.05	310.13	357.55	403.85
Carbohydrate calories (%)	38.64	27.88	19.79	22.77	13.99	07.01
Lipid calories (%)	18.76	35.02	47.44	19.18	35.65	48.40
Energy/Protein ratio (kcal/g protein)	10.56	12.13	13.73	07.75	08.93	10.09

[1]ICN Pharmaceuticals, Cleveland, Ohio, USA; [2]Loba Chemi, India

Table 2. Results of feeding test diets to C. mrigala fingerling during a 7-week growth trial

	Diets					
	I	II	III	IV	V	VI
Initial individual wet weight (g)	03.67 ±0.02	03.90 ±0.05	03.79 ±0.01	03.94 ±0.02	03.79 ±0.03	03.75 ±0.01
Final individual wet weight (g)	06.72 ±0.01	07.55 ±0.12	06.99 ±0.05	07.90 ±0.09	08.07 ±0.07	07.48 ±0.06
Percent increase in live weight* (g)	82.98^{a} ±0.99	93.34^{c} ±0.58	84.60^{a} ±1.59	100.51^{b} ±1.17	111.60^{d} ±0.30	99.39^{b} ±0.79
Feed conversion ratio*	03.35^{a} ±0.01	03.08^{b} ±0.00	03.22^{ab} ±0.01	02.89^{c} ±0.01	02.56^{d} ±0.00	02.85^{c} ±0.05
Protein efficiency ratio*	00.98^{a} ±0.00	01.07^{b} ±0.00	01.02^{a} ±0.01	00.85^{c} ±0.00	00.97^{a} ±0.00	00.87^{d} ±0.02
Protein productive value**	16.92^{a} ±0.06	16.59^{a} ±0.04	13.31^{b} ±0.22	15.14^{p} ±0.04	14.40^{p} ±0.01	10.09^{q} ±0.26
Energy conversion efficiency** (%)	14.98^{a} ±0.08	20.00^{b} ±0.08	19.28^{b} ±0.49	18.59^{p} ±0.30	22.28^{q} ±0.00	21.15^{q} ±0.38
Percent survival	96	91	96	100	97	94

Results are means ± SE of triplicate fish groups.

*Values in each row with same superscript are insignificantly different ($P < 0.01$)

**Since significant interaction effect of dietary energy and protein on energy and protein conversion efficiencies were detected, superscript letters represent comparison at the same protein level. Values in each row with same superscript are insignificantly different ($P < 0.01$)

Table 1. Details of fish size and stocking density

Species	Size groups				Stocking density (No. of fish/trough)	
	Group I		Group II			
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Group I	Group II
Labeo rohita	13.92 ±0.24	24.70 ±1.37	18.07 ±0.38	48.56 ±3.45	10	05
Cirrhinus mrigala	08.20 ±0.19	04.34 ±0.21	12.65 ±0.20	15.81 ±0.98	35	10

± SEM

Diet	Energy (kcal/100 g)	Moisture* (g/100 g, wet weight)	g/100 g, dry matter		
			Protein**	Fat**	Ash**
Initial		78.33 ±0.08	67.81 ±0.30	14.15 ±0.13	14.54 ±0.13
30% crude protein					
I	317.00	76.75^{a} ±0.33	67.76^{a} ±0.46	18.09^{a} ±0.42	11.61^{a} ±0.33
II	364.00	73.49^{b} ±0.29	56.63^{b} ±0.15	28.96^{bc} ±0.88	09.76^{bc} ±0.26
III	412.05	72.34^{b} ±0.21	50.16^{c} ±0.18	30.50^{bd} ±0.62	09.48^{c} ±0.08
40% crude protein					
IV	310.13	75.78^{p} ±0.16	66.61^{a} ±0.32	18.37^{a} ±0.02	11.48^{a} ±0.29
V	357.55	74.48^{pq} ±0.26	56.54^{b} ±0.03	28.30^{c} ±0.91	10.75^{ab} ±0.29
VI	403.85	73.60^{q} ±0.20	49.69^{c} ±0.44	31.63^{d} ±0.32	10.03^{bc} ±0.33
ANOVA					
Energy		S	S	S	S
Protein		N.S.	N.S.	N.S.	N.S.
Energy protein interaction		S	N.S.	N.S.	N.S.

Results are means ± SE of triplicate fish groups

*Since significant interaction effect of dietary energy and protein on body moisture content was detected, superscripts letters represent comparisons at the same protein level. Values in each column with same superscript are insignificantly different ($P < 0.01$)

**Values in each column with same superscript are insignificantly different ($P < 0.01$)

S = significant; N.S. = not significant, $P < 0.05$

Table 4. ANOVA of live weight gain and utilization efficiencies in C. mrigala fed experimental diet

Variables	Sources of variation	
Percent increase in live weight	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Feed conversion ratio	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Protein efficiency ratio	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Protein productive value	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	S
Energy conversion efficiency	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	S

N.S. = not significant; S = significant, $P < 0.05$

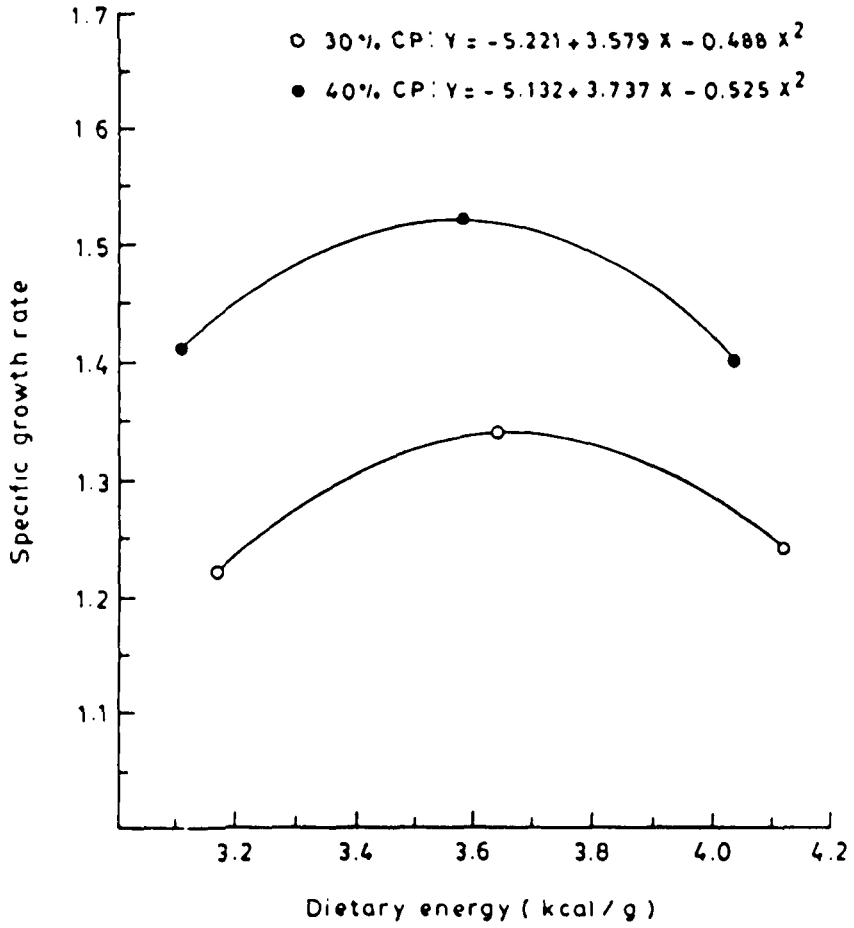

Fig. 1. Effects of dietary energy and protein on specific growth rate (%) of C. mrigala fed experimental diets.

CHAPTER 5

EFFECT OF DIETARY ENERGY AND PROTEIN LEVELS, AND THEIR INTERACTION, ON GROWTH, UTILIZATION EFFICIENCIES AND BODY COMPOSITION OF THE CATFISH, CLARIAS BATRACHUS (LINNAEUS)

INTRODUCTION

The knowledge of energy protein relationship, besides being an important prerequisite for the development of nutritionally adequate, economically viable and environmentally sustainable practical diet, provides a better estimate of both the energy and protein requirements of a species.

Of the two species of Clarias, inhabiting freshwaters of Asia, C. batrachus possesses characteristics that make it suitable for aquaculture. Although the fish is cultured, both traditionally and semi-intensively, in several Asian countries, including India, knowledge of its nutrition is far from complete. The available information deal mostly with its protein requirement, the reported values being highly variable ranging between 30-60% of the diet (AICRP, 1987; Chuapoehuk, 1987; and Khan and Jafri, 1990). Nutritional studies on African catfish, C. gariepinus are, however, well documented (Machiels and Henken, 1985; Henken et al., 1986; Huisman and Richter, 1987). The dietary protein requirement in fish is dictated, besides other factors, by a delicate balance of dietary

energy to protein ratio.

In the present study, the effect of dietary energy and protein, and their interaction, have been examined on the growth, utilization efficiencies and body composition of C. batrachus.

MATERIALS AND METHODS

Preparation of experimental diets

Diets were formulated to contain 25, 35 and 40% crude protein, each with two levels of metabolizable energy (360.3 ± 5.72 and 407.1 ± 4.40 kcal/100 g), using semipurified ingredients, in a 3X2 factorial design of experiment. Diet containing 50% C.P. and 407.1 kcal/100 g was employed as control (Table 1). Dietary energy was calculated using fuel values as given elsewhere (page 23). At each protein level, two different levels of lipid (8 and 16%) were maintained and the desired dietary energy levels achieved by varying the dextrin and α-cellulose components. The energy to protein (E/P) ratio in the experimental diets ranged between 8.88-16.52 kcal/g protein. The method of preparation of diet was similar to that described under General Methodology section (page 13).

Feeding trial

Details of acclimation and experimental set up were the same as mentioned elsewhere (page 12). Young C. batrachus (11.80 ± 00.17 cm; 9.44 ± 00.06 g) were selected from the acclimated

fish lot and stocked in 70 l circular polyvinyl troughs (water volume 55 l) supplied with continuous flow (1 l/min) of ground water. Ten fish, with three replicates, were randomly fed the control or one of the six experimental diets. Differences in total initial weight of fish in various groups were insignificant ($P < 0.05$). The experiment was continued for 5 weeks. Average water temperature over the experimental period was 25 ± 1°C.

The fish were fed to satiation at 0900 and 1700 h. Uneaten feed, if any, was collected over a fine mesh sieve and dried (100°C) for calculating the quantity of feed consumed. The mean feed consumption (appetite) $fish^{-1}$ was calculated, dividing total feed intake by number of $fish\ trough^{-1}$, over the experimental period. Other experimental details, remained the same as given under General Methodology section (page 13-14).

Performance of diets, in terms of weight gain and nutrient utilization, was assessed using standard definitions (page 19-21).

Gross energy and proximate analyses

For the initial gross energy and proximate analyses, fish were randomly taken out from the acclimated stock, sacrificed, skinned and their trunk muscle scraped. The estimates were made in triplicate using standard methods of analysis (page 14-18). At the end of the trial, required number of fish from each trough were similarly slaughtered for the analyses of their final gross energy and body composition.

Statistical analyses

Data on growth parameters, feed consumption, utilization efficiencies and body composition were subjected to factorial (dietary energy and protein density) analysis of variance, for the six experimental diets, to test the effect of energy and protein, and their interaction.

Analysis of variance was also performed on data for the control and experimental diets. When significant differences were detected, with no significant interaction between dietary energy and protein, the treatment means were compared using Duncan's new multiple range test (Duncan, 1955). On appearance of a significant interaction, the various treatment means were compared only at either of the isocaloric groups.

Relationships between spcecific growth rate (SGR %) vs. E/P ratio and dietary protein vs. weight gain (%) were established by linear regression analysis (Snedecor and Cochran, 1967).

RESULTS

Results of feeding C batrachus with varying levels of energy and protein are summarized in Table 2 and 3. A significant influence of energy and protein was evident on growth, in terms of percent live weight gain (Table 4). Evidence of energy- protein interaction on weight gain was not seen. A general decline in live weight gain was noted with increasing dietary energy. Within

isocaloric diets, percent weight gain was found directly related (360.3 kcal/100 g : $r = 0.97$, $P < 0.1$, $n = 3$; and 407.1 kcal/100 g : $r = 0.99$, $P < 0.01$, $n = 4$, including control) to dietary protein levels (Fig. 1). However, diet containing 360.3 kcal/100 g always produced better growth, the maximum being at 40% crude protein level, against diets containing 25, 35 or 40% protein with 407.1 kcal/100 g. Specific growth rate (SGR %) registered a linear negative correlation (Fig. 2) with E/P ratios (360.3 kcal/100 g : $r = -0.97$, $P < 0.025$, $n = 4$, including a statistical growth rate data obtained for 50% C.P.; and 407.1 kcal/100 g : $r = -0.99$, $P < 0.005$, $n = 4$, including the control diet). A significant effect of energy, protein, and their interaction was noticeable on SGR (%).

The mean feed consumption (mg/fish day^{-1}) was found to be influenced both by energy and protein contents of the diet as well as by their interaction (Table 4). In general, at a given protein level, feed consumption was found to reduce with increase in dietary energy. Influence of dietary protein on feed consumption was positively linear (360.3 kcal/100 g : $r = 0.99$, $P < 0.05$, $n = 3$ and 407.1 kcal/100 g : $r = 0.93$, $P < 0.10$, $n = 3$) at a given energy level. Correlation analysis between E/P ratios and feed consumption indicated a highly significant negative linear effect ($r = 0.96$, $P < 0.005$, $n = 6$). Fish fed 40% crude protein in low (360 kcal/100 g) energy group consumed maximum (830.74 mg/fish day^{-1}), while the minimum (646.11 mg/fish day^{-1}) being at 25% crude protein with high (407.1 kcal/100 g) dietary energy content. The percent live weight gain (Y) showed a positive correlation with daily mean feed

consumption (X), the relationship followed the equation Y = -138.368 + 0.399 X ($r = 0.94$, $P < 0.005$, $n = 6$).

Feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy conversion efficiency (ECE) were influenced both by energy and protein in diet (Table 4). With the exception of PPV, the interaction effect of energy and protein on the above parameters was insignificant. Among the experimental diets, maximum (1.56) feed conversion was noted in the group fed 40% crude protein and low energy. At a given energy level, FCR improved with decreasing (360.3 kcal/100 g: $r = -0.99$, $P < 0.05$; and 407.1 kcal/100 g: $r = -0.96$, $P < 0.05$) E/P ratios. Increasing dietary protein, within the isocaloric diets, improved FCR ($P < 0.05$). At each protein level, poor FCR and lower values of PER, PPV and ECE occurred when energy content increased from low to high. Both PER (360.3 kcal/100 g: $r = -0.99$, $P < 0.05$; and 407.1 kcal/100 g: $r = -0.97$, $P < 0.01$) and PPV ($r = -0.99$, $P < 0.05$, at both the energy levels) showed inverse relationship with increasing dietary protein, while ECE followed an opposite trend ($r = 0.99$, $P < 0.05$, at both the energy levels), when isocaloric diets were compared. Protein deposition (PPV) and conversion (PER) were maximum (35.8 and 2.17, respectively) at the lowest protein (25%) level with 360.3 kcal/100 g diet, while highest ECE (25.03) amongst the experimental diet was observed at 40% crude protein with similar level of energy.

Moisture, crude protein and lipid were found to vary significantly among the various treatment groups. Significant influence of dietary energy was noticeable on these variables. Effect of

dietary protein levels or energy-protein interaction was insignificant on body composition (Table 3). At the end of the feeding trial all the experimental groups, including the control, exhibited higher percentage of lipid and lower percentages of body moisture, protein and ash than their initial values. The percent body lipid increased with dietary energy. Moisture, protein and ash maintained an inverse trend with dietary energy. These variables also showed a negative relationship with body lipid.

DISCUSSION

Significant influence of dietary energy and protein on the growth of C. batrachus indicates that, among the various combinations of dietary energy and protein, 360.3 kcal/100 g with 40% C.P. (E/P ratio 8.88) matches the requirement of the species for maximum weight gain (%). Further reduction of E/P ratio to 8.13, as contained in the control diet, produced insignificant gain as compared to the above energy-protein combination. SGR (%) when plotted against E/P ratios (Fig. 2) at each energy level also intersected at or near an E/P ratio of 8.88. The observed negative correlation between E/P ratio and SGR (%) indicates that higher E/P ratios in diet do not prove beneficial to fish in terms of growth. The negative correlation between E/P ratio and the amount of feed consumed justifies the above fact. The amount of feed consumed appears to have a direct bearing on weight gain, and this can be understood from the equation describing a positive correlation between percent live weight gain and daily mean consumption. Page and Andrews (1973), and Lovell

(1979), while examining energy-protein relationship in channel catfish, observed that high digestible energy to protein ratio causes cessation of feeding, before sufficient protein is consumed, the consumption being primarily determined by the total available energy in the diet. These authors have attributed the decreased growth of channel catfish to reduced consumption at higher dietary energy density. Lee and Putnam (1973) also opined that, in rainbow trout, feed intake is regulated by its calorie content. Several other workers (Millikin, 1983; Beamish and Medland, 1986; and Ellis and Reigh, 1991) have confirmed the above fact in various other fish species.

A general reduction in percent weight gain with increased energy content in diet, and a direct relation with dietary protein content observed in C. batrachus, conform with the findings on red drum (Daniels and Robinson, 1986). In the experiment with an omnivorous fish, C. mrigala, fed various energy densities in iso-nitrogenous (40% C.P.) diet, a quadratic growth pattern was noted in relation to dietary energy levels (Chapter 3). In the present study, since only two energy levels were used, quadratic function of energy density could not be established although reduction in growth rate with increased energy intake was found similar to that observed in C. mrigala. The optimum value (8.88) for E/P ratio was found identical in the two species. This value also fell close to the requirement of 8-9 kcal g^{-1} of protein recommended for common carp and channel catfish (Lovell, 1984).

The insignificant interaction effect of dietary energy and protein on live weight gain (%), as observed in C. batrachus, was

also reported in striped bass (Millikin, 1983), Arctic charr (Tabachek, 1986), Walleyes (Barrows et al., 1988) and red drum (Serrano et al., 1992).

The significant influence of energy and protein content of the diet, and their interaction on daily mean feed intake revealed the importance of these variables (energy and protein) in dietary formulation. However, the data indicate that, irrespective of protein level, feed consumption gets reduced with increasing energy level. Influence of protein content on feed consumption of C. batrachus was significant and more pronounced in the group fed low energy diet. Effect of protein level and calorie density on feed consumption has also been reported in other fishes (Lee and Putnam, 1973; Page and Andrews, 1976; Marais and Kissil, 1979; Jobling and Wandsvik, 1983; Tabachek, 1986; Wang et al., 1985; De Silva and Gunasekera, 1989 and De Silva et al., 1991).

Factorial analysis of variance showed influence of both dietary energy and protein on FCR. The overall performance of the diet, in relation to weight increment, can be judged from feed-gain ratio (FCR). However, the positive association between dietary protein and feed conversion indicates that the highest protein level might not have reached in the present experiment. But multiple range analysis of data showed FCR values levelling off at 35% dietary protein incorporation, the maximum FCR obtaining at 40% C.P. with an appropriate (360.3 kcal/100 g) combination of dietary energy. This may thus be taken as the optimum range (35-40% C.P.) of dietary protein required for better conversion. Irrespective of

dietary protein level, poor FCR with increasing dietary energy seems related to reduced consumption at higher energy level. In channel catfish, however, Page and Andrews (1973) have reported improvement in FCR with dietary energy. This exception may be explained by the fact that the energy level used by these workers for channel catfish was well below the level employed for C. batrachus.

In the fishes, besides other factors, protein utilization is known to depend on quality and level of both protein and energy in the diet (Steffens, 1981). In C. batrachus, dietary protein content exerted a negative influence on PER and PPV, the highest values of these being at the lowest (25%) crude protein level that neither supported growth for produced better FCR. Identical trends have been noted in other fishes (Ogino and Saito, 1970; Adron et al., 1976; Dabrowski, 1977; Reinitz et al., 1978; Parazo, 1990). An exception was, however, seen in the work of Zeitoun et al. (1973), and Yu et al. (1977) who observed high PER and maximum growth in rainbow trout at relatively high dietary protein level. Increased energy intake within isonitrogenous diets resulted in reduced PER and PPV in C. batrachus. A corollary to this finding was seen in the work of Page and Andrews (1976), and Machiels and Henken (1985) on channel catfish and African catfish, respectively. Increasing energy concentration per unit amount of protein(E/P ratio), within isocaloric diets, showed a positive correlation with PER and PPV. This seems to indicate that more the energy available g^{-1} protein greater is the utilization of protein for growth, measured in terms

of PER and PPV. The above fact finds support from the negative relationship observed, within isocaloric diets, between E/P ratio and energy deposition (ECE). Low energy conversion at high E/P ratio suggests that more energy is utilized to meet the energy demands of the body and only little get deposited (low ECE). The increasing trend of energy deposition which plateaued at 35% C.P., seems to indicate that a crude protein level between 35-40%, with an energy density of 360.3 kcal/100 g, in the diet adequately meets the protein and energy requirements of C. batrachus. High ECE in fish fed the control diet points to the fact that contained energy and protein levels are surplus and this may be considered as nutritionally and economically wasteful. On the basis of weight gain, and levelling-off in FCR, PER, PPV and ECE values, it may be surmised that 35-40% C.P. and 360.3 kcal/100 g in diet be taken as optimum for C. batrachus. Khan and Jafri (1990) have also indicated that 40% C.P. in the diet meets the requirement of this species.

Contrary to the observations of Sabaut and Luquet (1973), and Marais and Kissil (1979) on Sparus aurata, the data on body composition of C. batrachus reveal that dietary energy level significantly influence the various body constituents (Table 3). The concomitant increase observed in body lipid with dietary energy, has also been reported in other fish species (Watanabe, 1982; and Zeitler et al., 1984). The inverse relationship noted between dietary energy (lipid) and body moisture, protein or ash has likewise been pointed out by other workers (Parazo, 1990 and El-Sayed and Teshima, 1992), who attributed this to a diluting effect of increased

body lipid. This assumption gains further support from the negative relationship maintained by body lipid with moisture, protein and ash content of the muscle in C. batrachus. The range of dietary protein tested during the present study, for its possible effect on body composition, demonstrated no influence on any of the above variables. The finding seems in agreement with the observations of Page and Andrews (1973), Reinitz et al. (1978), Millikin (1982), Daniels and Robinson (1986), and De Silva et al. (1991), but contradicts that of Lee and Putnam (1973) and Tabachek (1986). However, Millikin (1983) found a direct association of body protein with dietary protein when trial was continued for a longer period. Similar to the observations of Barrows et al. (1988) and El-Sayed and Teshima (1992) on other fishes, energy-protein interaction effect on body composition remained insignificant in C. batrachus.

The present study thus clearly demonstrates the importance of dietary energy-protein relationship on fish growth, feed/nutrient utilization efficiency and body composition. To produce sufficient weight gain and better feed conversion, the diet for C. batrachus should contain 35-40% crude protein and 360.3kcal/100 g of energy. This level of protein could further be lowered to 25% C.P. with the above calorie content, to reduce feed cost. A reduction in growth with low protein diet (25% C.P.) is compensated by relatively high protein retention. Therefore, based on the purpose of farming, while formulating feed for C. batrachus, a compromise is suggested between growth rate and cost effectiveness of the diet.

SUMMARY

Young C. batrachus(11.80±0.17cm) were fed semipurified diets containing three levels of crude protein (25, 35 and 40%, as fed), each at two levels of metabolizable energy (360.3±5.72 and 407.1±4.40 kcal/100 g), for evaluating the effect of dietary energy and protein, and their interaction on growth, utilization efficiencies and body composition, in a flow-through system. Over the 5-week feeding trial at 25±1°C, growth of fish, in terms of live weight gain (%), increased with increasing dietary protein ($r = 0.95$; $P < 0.05$) at both the energy levels. A general reduction in growth rate occurred with increase in energy density of diets at each protein level. Within isocaloric diets, SGR (%) exhibited a linear negative correlation ($r = -0.96$; $P < 0.10$ for 360.3kcal and $r = -0.99$; $P < 0.005$ for 407.1kcal/100 g) with E/P ratios. Mean feed consumed was found influenced by dietary energy and protein levels and their interaction. FCR, PER and ECE were affected only by dietary energy and protein levels while PPV was influenced both by the levels of energy and protein as well as their interaction. When isocaloric diets were compared, PER and PPV exhibited a positive, whereas FCR and ECE indicated an inverse correlation with E/P ratios. Carcass composition was influenced by dietary energy but influence of dietary protein or energy-protein interaction was not evident.

Table 1. Ingredient and proximate composition of experimental diets

Ingredients (g/100 g, as fed)	Diets						
	I	II	III	IV	V	VI	Control
Casein (Vitamin free; 84.0% C.P.)[1]	22.32	22.32	31.25	31.25	35.71	35.71	44.64
Gelatin (87.6% C.P.)[2]	07.13	07.13	09.98	09.98	11.41	11.41	14.26
Dextrin	53.16	47.16	38.16	32.33	30.66	24.66	13.00
Corn oil	05.34	10.64	05.34	10.64	05.34	10.64	10.64
Cod liver oil	02.66	05.33	02.66	05.33	02.66	05.33	05.33
Vitamin mix.	01.00	01.00	01.00	01.00	01.00	01.00	01.00
Mineral mix.	03.00	03.00	03.00	03.00	03.00	03.00	03.00
α-cellulose	03.39	01.39	06.61	04.44	08.22	06.22	06.10
Carboxymethyl cellulose	02.00	02.00	02.00	02.00	02.00	02.00	02.00
Proximate composition % (calculated)							
Crude protein	25.00	25.00	35.00	35.00	40.00	40.00	50.00
Crude fat	08.00	16.00	08.00	16.00	08.00	16.00	16.00
Carbohydrate	53.00	47.00	38.00	32.00	31.00	25.00	13.00
Metabolizable energy (kcal/100 g, as fed)	366.56	413.00	359.06	406.65	355.31	402.31	406.50
Carbohydrate calories (%)	50.75	39.96	37.19	27.82	30.20	21.45	11.19
Lipid calories (%)	18.55	32.92	18.93	33.44	19.13	33.80	33.45
Energy/protein ratio (kcal/g protein)	14.66	16.52	10.25	11.61	08.88	10.57	08.13

[1]ICN Pharmaceuticals, Cleveland, Ohio, USA; [2]Loba Chemi, India

Table 2. Results of feeding experimental diets with varying E/P ratios C. batrachus during a 5-week growth trial.

	Diets						
	I	II	III	IV	V	VI	Control
Initial individual wet weight (g)	09.53 ±0.01	09.28 ±0.05	09.77 ±0.10	09.57 ±0.03	09.38 ±0.12	09.29 ±0.02	09.25 ±0.06
Final individual wet weight (g)	23.39 ±0.24	20.72 ±0.06	26.39 ±0.04	25.07 ±0.16	27.97 ±0.24	26.22 ±0.20	28.37 ±0.28
Percentage gain in live weight *	145.49^{a}	123.21^{b}	170.09^{c}	162.05^{c}	198.10^{d}	182.27^{e}	206.50^{d}
Specific growth rate	02.56 ±0.03	02.29 ±0.02	02.83 ±0.02	02.75 ±0.02	03.11 ±0.01	02.96 ±0.01	03.19 ±0.01
Feed consumed (mg/fish day^{-1})**	729.59^{a} ±14.25	646.11^{p} ±09.40	786.92^{b} ±05.68	775.35^{q} ±07.72	830.70^{c} ±14.66	770.91^{q} ±06.89	730.48^{r} ±06.02
Feed conversion ratio*	01.83^{a} ±0.01	01.97^{b} ±0.04	01.65^{c} ±0.01	01.75^{d} ±0.03	01.56^{c} ±0.04	01.59^{c} ±0.03	01.33^{e} ±0.01
Protein efficiency ratio*	02.17^{a} ±0.01	02.02^{b} ±0.04	01.72^{c} ±0.01	01.63^{d} ±0.03	01.60^{d} ±0.03	01.57^{d} ±0.03	01.49^{d} ±0.04
Protein productive value (%)**	35.80^{a} ±0.04	31.17^{p} ±0.83	29.16^{b} ±0.10	27.13^{q} ±0.67	25.43^{c} ±0.64	24.83^{r} ±0.53	23.38^{r} ±0.13
Energy conversion efficiency*	19.43^{a} ±0.01	16.98^{b} ±0.42	23.72^{c} ±0.10	22.13^{d} ±0.51	25.03^{c} ±0.61	24.41^{c} ±0.34	29.79^{e} ±0.15
Percent survival	94	100	96	90	96	94	100

Results are means ± SE of triplicate fish groups.

*Values in each row with same superscript are insignificantly different ($P < 0.01$)

**Since significant interaction effect of dietary energy and protein on feed consumed and protein productive value were detected, superscript letters represent comparison at the same energy level. Values in each row with same superscript are insignificantly different ($P < 0.01$).

Table 3. Body composition of C. batrachus fed experimental diets

Diet	Energy (kcal/100 g)	Moisture* (g/100 g, wet weight)	g/100 g, dry matter		
			Protein*	Fat*	Ash
Initial		80.64 ±0.22	74.29 ±0.53	05.86 ±0.12	07.16 ±0.00
25% crude protein					
I	366.56	77.96^{a} ±0.32	70.94^{a} ±0.92	17.39^{a} ±0.53	05.87 ±0.07
II	413.00	77.87^{ab} ±0.01	67.53^{bc} ±0.09	21.76^{b} ±0.63	5.54 ±0.12
35% crude protein					
III	359.06	77.54^{ab} ±0.17	71.19^{a} ±1.06	16.99^{a} ±0.27	05.83 ±0.16
IV	406.65	76.39^{ab} ±0.43	66.78^{bc} ±0.01	20.56^{c} ±1.01	05.41 ±0.12
40% crude protein					
V	355.31	78.34^{ab} ±0.02	71.08^{a} ±0.02	16.86^{a} ±0.82	05.95 ±0.14
VI	402.31	77.34^{ab} ±0.31	67.60^{b} ±0.28	22.82^{d} ±0.17	05.62 ±0.06
Control	406.50	76.79^{b} ±0.15	65.65^{c} ±0.26	19.63^{e} ±0.13	05.67 ±0.07
ANOVA					
Energy		S	S	S	S
Protein		N.S.	N.S.	N.S.	N.S.
Energy protein interaction		N.S.	N.S.	N.S.	N.S.

Results are means ± SE of triplicate fish groups.

*Values in column with same superscript are insignificantly different ($P < 0.01$)

S = significant; N.S. = not significant, $P < 0.05$

Table 4. ANOVA of live weight gain, feed consumption and utilization efficiencies in C. batrachus fed experimental diets

Variables	Sources of variation	
Percentage gain in live weight	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Specific growth rate	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	S
Total feed consumed	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	S
Feed conversion ratio	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Protein efficiency ratio	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Protein productive value	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.
Energy conversion efficiency	Dietary energy	S
	Dietary protein	S
	Energy-protein interaction	N.S.

N.S. = not significant; S = significant, $P < 0.05$

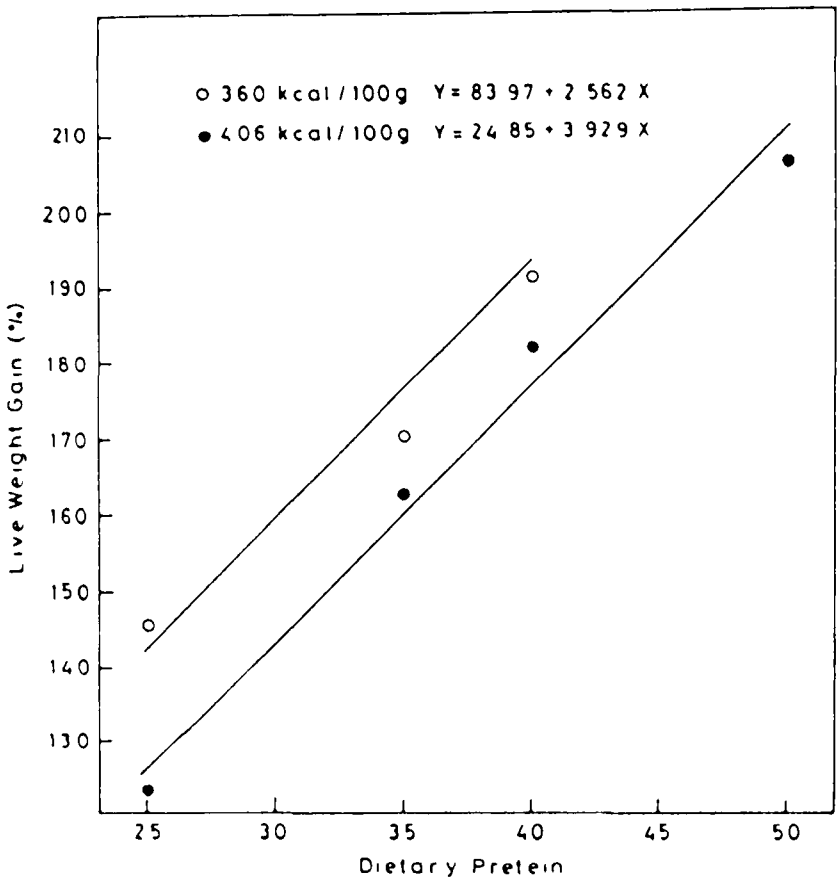

Fig. 1. Effects of dietary protein levels on live weight gain of C. batrachus fed experimental diets.

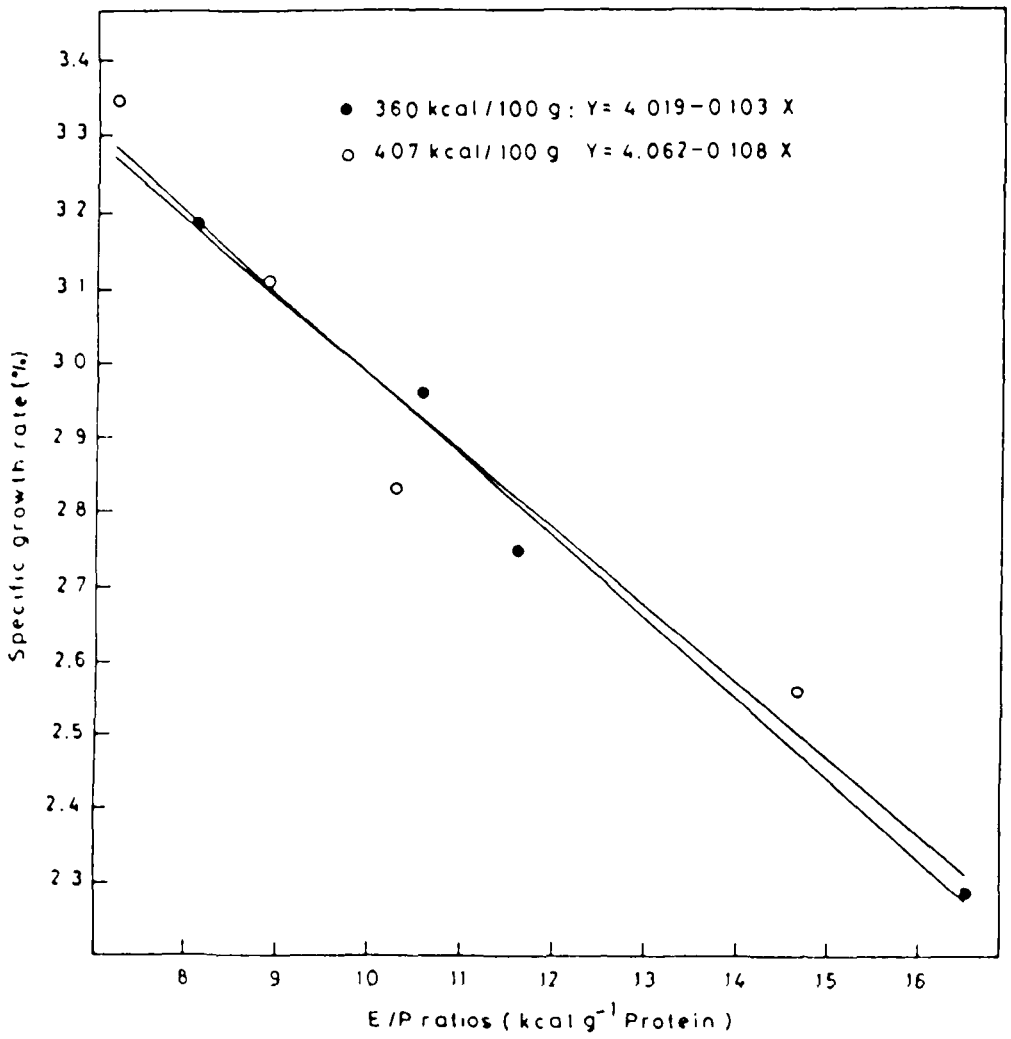

Fig. 2. Effects of dietary E/P ratios on specific growth rate (%) of C. batrachus fed experimental diets.

PART II

CHAPTER 6

ANNUAL VARIATIONS IN ENERGY STORAGE AND BIOLOGICAL INDICES OF ADULT CATFISH HETEROPNEUSTES FOSSILIS (BLOCH)

INTRODUCTION

Temperate fish species are reported to show marked changes in their somatic growth, tissue chemistry and energy storage in relation to factors like changes in food availability, intensity of feeding and energy expenditure (Medford and Mackay, 1978; Pierce et al., 1980; Adams et al., 1982 and Dygert, 1990). In tropical region, where environmental variations are not so distinct, such changes in fish have been attributed more to their inbuilt rhythm - the reproductive cycle (Love, 1980). The need for understanding body composition changes in tropical fish had long been felt, and considerable amount of information is available from the work of Jafri (1965).

Heteropneustes fossilis, one of the common and popular catfishes of India, is well distributed in freshwater swamps and marshy land of the country. Because of its nutritional qualities, the fish has a high marketability. The growth, length-weight relationship, feeding and breeding of this fish have been studied in detail (Ghosh and Kar, 1952; Qasim and Qayyum, 1961; Bhatt, 1968; Thakur and Das, 1974, and Kohli and Goswami, 1986). However, correlative studies on energy changes and biology of the fish seem

lacking. Shreni (1980) has reported seasonal variations in the chemical composition of H. fossilis.

This study attempts to establish relationships between energy storage and various biological indices of H. fossilis seasonally.

MATERIALS AND METHODS

Representative samples of H. fossilis were obtained from local ponds of Aligarh (Lat. 27°34'30"N. Long. 78°4'26"E) at regular monthly intervals over a period of one year (March, 1988 to February, 1989). Since male and female of this species attain first maturity at 5.5 and 12 cm (total length), respectively (Talwar and Jhingran, 1991), specimens beyond the above lengths were taken up for the study. Before obtaining the tissue samples, each fish was weighed (g) and its total length (cm) measured. They were then dissected out and sexed. Wet weights (g) of gut, liver and gonads were separately recorded. The data were utilised for calculating the various biological indices (page 21).

Fish were sacrificed until enough gut, liver and gonad samples were collected. Peritonial fat was carefully removed from the gut. Muscle was scraped from mid-dorsal region of the body. Pooled samples of each tissue was used for gross energy determination (page 18). In the case of testis, where quantity of sample was inadequate, a known amount of benzoic acid was added

to produce a measurable amount of heat.

Data for each month are mean ± S.E. One way analysis of variance was employed to test seasonal variation, and the significance tested through Student's t-test.

RESULTS

A total of 79 male and 91 female specimens, with an average size of 18.3±2.5 cm, respectively, were sampled over the period for studying the changes in biological indices, and calorific values in various tissues (Table 1-2).

Biological indices

Analysis of variance showed a highly significant influence of months on condition factor (K) only in female fish (male: $F = 2.49$; d.f. = 11,67; P 0.01; and female: $F = 8.46$; d.f. = 11,79; $P < 0.01$). The seasonal cycle in condition factor was also better defined and significantly higher ($P < 0.05$) in female, particularly during April to August. The maximum 'K' values for male and female fish were obtained in May and June, respectively (Fig. 1).

Influence of months on gastro-somatic index was also found significant (male: $F = 7.94$; d.f. = 11,67; $P < 0.01$; and female: $F = 20.91$; d.f. = 11,79; $P < 0.01$), with both male and female fish following almost similar pattern of changes (Fig. 2). Two peaks of gastro-somatic index were noted, the first occurring in March and the other from August-November. The values started gradually declining from April onwards, reaching the all time low in July.

The liver-somatic index also showed variations with season, and the changes were significant (male: $F = 8.40$; d.f. = 11,67; $P < 0.01$; and female: $F = 4.70$; d.f. = 11,79; $P < 0.01$). From lowest value in March, the liver-somatic index gradually increased to the maximum in August and September in male and female fish, respectively. However, after a fall in October, the fish maintained almost constant values till January. During this period the female fish maintained higher liver-somatic index than the male (Fig. 3).

Changes in gonado-somatic index in both the sexes were characterized by significant (male: $F = 106.90$; d.f. = 11,67; $P < 0.01$; female: $F = 123.30$; d.f. = 11,79; $P < 0.01$) influence of months, exhibiting well-defined peaks in the month of July. In the two sexes, the gonads started differentiating from April and this was accompanied by more rapid changes in their gonado-somatic index. From August onwards, the values started declining, approximating to their pre-spawning level in October, and remaining unchanged in the rest of the months (Fig. 4).

The various indices, excepting the gonado-somatic index, showed insignificant ($P < 0.01$)differences between the sexes.

Calorific value

Seasonal influence on muscle calorie content was found significant (male: $F = 9.04$; d.f. = 11,32; $P < 0.01$ and female: $F = 13.90$; d.f. = 11,33; $P < 0.01$). Two peaks of energy accumulation were evident in muscle, one in June and the other

in November-December. The lowest calorific value was recorded in August (Fig. 5).

Monthly variations in calorie content of gut were also significant (male: $F = 5.97$; d.f. = 11,24; $P < 0.01$; and female: $F = 10.92$; d.f. = 11,24; $P < 0.01$). After a peak in February, the gut calories declined gradually to the minimum during May-July. This was followed by an increment and the peak occurred in November. A small depression in energy content of the gut was, however, noted during December-January (Fig. 6).

Monthly calorific values of liver tissue showed significant (male: $F = 4.00$; d.f. = 11,24; $P < 0.01$; and female: $F = 12.47$; d.f. = 11,24; $P < 0.01$) fluctuations. Like muscle, the liver also showed two peaks of energy accumulation, one in May and the other in December/January. The lowest value was recorded in October (Fig. 7).

The variations in the energy content of muscle, gut and liver were more or less identical in the two sexes.

Well-defined and significant ($F = 7.31$; d.f. = 11,24; $P < 0.01$) monthly variations were found in ovarian calorie content. The changes were characterized by a steep increase from March, culminating in a peak in July, and a gradual fall to its minimum in February (Fig. 8).

Influence of months on testicular energy changes were also significant ($F = 215.65$; d.f. = 11,24; $P < 0.01$). Testicular tissue showed a rapid increase in its calories from March to July followed

by a steep decline to pre-spawning level in October. The testicular calorific value, however, remained significantly ($P<0.05$) lower, even during its peak period in July, than the ovarian calories (Fig. 9).

DISCUSSION

The pattern of changes in GSI and gonadal calories of H. fossilis demonstrate that the fish is an annual breeder. Peak gonado-somatic index, accompanied by maximum deposition of calories in gonads in July, and a sudden depletion in subsequent months, suggest that the fish reaches its peak maturity during July. The deposition of calories in ovary during peak maturity ensures sufficient energy store for the initial nourishment of spawn. Ghosh and Kar (1952) have also observed highest gonadal activity in female H. fossilis during the month of July, while maximum spawning of the fish has been reported in August-September (Qasim and Qayyum, 1961).

The calorie content (5.92 kcal g^{-1}) of ripe ovaries in H. fossilis was found higher than the mean (5.61 kcal g^{-1}) value quoted by Wootton (1979) for the ripe ovaries of 50 other teleostean species. The energy content (4.90 kcal g^{-1}) of testes in this fish, just prior to spawning, was significantly lower than that of the ovaries in similar condition. In addition, it has been observed that in fully ripe fish, the ovaries form up to 23% of the total body weight, whereas, the testes form less than 1% of the body weight, the latter being only 3.58% of that of the ovary. It may therefore be assumed that, out of the three-fold cost of reproduction, namely, primary

sex products, secondary sexual characters and reproductive behaviour, the male H. fossilis spends more calories towards reproductive behaviour, including courtship, care of eggs and newly hatched fry, territoriality, etc., since the fish does not develop any secondary sexual characters. A corollary to this assumption is seen in the observations of other workers (Wootton, 1985 and Rogers, 1986), who have noted similar disparity in gonadal calorific value of the two sexes.

Jafri and Khawaja (1968) have established that in several tropical fishes the biochemical composition of tissue is greatly influenced by two main features of biology, namely, feeding (nutrition) and maturation cycle. The influence of months on gastro-somatic index, as observed in H. fossilis, may be related to both maturation cycle and the feeding activity of the fish. The gradual declining trend in the gastro-somatic index from March onwards coincided with the onset of differentiation of gonadal tissue in April. A rapid increase in gonado-somatic index, with a peak in July, which corresponded to minimum gastro-somatic index, points to a gradual decline in feeding activity of the maturing fish. Pierce et al. (1980) observed minimum feeding activity, prior to and during spawning, in gizzard shad (Dorosoma cepedianum). However, the present observation contradicts the finding of Hislop (1984) and Pulliainen and Korhonen (1990), who reported increase in food intake at the time of gonad growth in Merlangius merlangus and Lota lota, respectively. Further, it appears that, to compensate somatic loss

as a result of breeding activity, H. fossilis resumes active feeding soon after spawning, as indicated by increasing values of gastro-somatic index. Bhatt (1968), while studying the biology of H. fossilis, observed maximum feeding intensity during monsoon month (September) and lowest during June. Lower gastro-somatic index during winter (December-January) may be related either to low environmental productivity or to less active feeding by fish due to low temperature or both.

The pattern of changes in the calorific value of gut, with the values becoming minimum during May-July and maximum in November/December, may provide further support to the above assumption. A small depression in the gut calorific value from December onwards, coincided with low feeding rate during this period.

To meet the energy demand during breeding season, when feeding activity is low, liver may be over active in rapid generation and mobilization of energy nutrients. In female fish, the chief constituent of egg yolk, vitellogenin, is also synthesized in the liver. Thus, it appears that liver passes through its most active period during April to August/September which causes its enlargement, leading to high liver-somatic index in these months. As would be evident from the changes in gonado-somatic index, the fish spawn in August/September, but its impact on liver-somatic index was reflected only during October when most fish were in spent condition. During winter months, December onwards, when

feeding activity gets reduced, a reduction in the metabolic activity of liver might occur, leading to low liver-somatic index, and the impact continues till March.

Variations in the calorific value of liver may also be linked to feeding rhythm. The fish in its post-spawning period, October onwards, indulges in active feeding, resulting in a steep rise in liver calorific value, with greater synthesis and storage of lipid reserves. It has been observed in many fishes that energy stored as growth can in turn be greatly influenced by changes in body composition, particularly by lipid content (Medford and Mackay, 1978). The work of Shreni (1980), who noted peaks of fat accumulation in the liver of H. fossilis during pre- and post-spawning months, seems to support the above observation. The reduced feeding activity during winter months could not influence liver calories instantaneously, although liver calories started depleting from January. The increasing trend of liver calorific values from March to May, may be considered as the 'preparatory phase'. The reserves accumulated in the liver during this period ultimately start channelising towards gonadal maturity, as revealed by a decline in the liver calorific value.

Low intensity of feeding, reflected by gastro-somatic index and gut calories, becomes apparent as the fish reaches its peak maturity. Interestingly, coinciding with gonad maturity, the calorific value of muscle increased till June, and this can be compared with the so-called 'preparatory phase' of the liver. The increased

calorific value in both muscle and liver during low feeding intensity may possibly be attributed to some nutrient inter-conversion processes operating in these tissues. Since lipid contains almost twice as much gross energy as protein and carbohydrate, the conversion of the latter two nutrients to lipid will directly influence the calorie content of the body tissue. The two peaks of muscle lipid accumulation noted in the fish during May and November by Shreni (1980) strengthens the above assumption. Mobilization of energy nutrients from muscle, as also from liver, in subsequent months (May onwards) get utilized mostly for gonad development, thereby depleting the muscle/liver calories. This becomes evident, since gonads were the only tissue found gaining energy during this period. Accumulation of energy reserves in somatic tissues in the pre-spawning period and their mobilization for gonad development have also been reported in stickleback, Gasterosteus aculeatus (Wootton, 1979), Norweigian cod, Gadus morhua (Eliassen and Vahl, 1982), and Pacific cod, Gadus macrocephalus (Smith et al., 1990). Needham (1964) has concluded that growth of gonad and its differentiating process are energy consuming.

Muscle calories in H. fossilis were found most depleted in the spawning month (August). Thereafter, on the resumption of active feeding, the muscle appeared to start accumulating calories again and this corresponds to the second peak (in November) of muscle lipid deposition reported by Shreni (1980). However, reduced feeding rate during winter showed an instantaneous and marked influence on muscle calorific value.

Maturity cycle, as indicated by changes in gonadosomatic index, precisely coincided with changes in gonadal calorie. The steep fall in testicular calorific value in the spawning months (August/September) indicate towards complete ejaculation of its products to ensure maximum fertilization. A gradual decline in ovarian calories during this period, on the other hand, suggests that small-sized or incompletely developed eggs, which could be wasted unfertilized, were perhaps not released, but gradually resorbed showing a decreasing slope of energy loss.

Interestingly, only in female, condition factor (K) exhibited significant variations with change in season. These variations may be related more to spawning than feeding rhythm. This is understandable because in female, although the denominator (length) in definition remained almost constant, the 'K' value continued to increase gradually. It may thus be stated that enlargement of gonad (increase in volume and weight) with the onset of maturation cycle (April) causes increment in 'K' value. This observation, however, disagrees with that of Bhatt (1968), who attributed monthly fluctuations in 'K' value of H. fossilis more to its feeding rhythm.

The relationship stablished between seasonal changes in various biological indices and calorific value of somatic and gonadal tissues of H. fossilis will be helpful in developing strategy for the rational management of aquaculture of this important catfish species.

SUMMARY

Changes in annual calorific value of various somatic and gonadal tissues, and biological indices of mature male and female catfish, H. fossilis, collected each month, over a period of one year, from northern part (Aligarh) of India, are described and discussed. The biological indices studied, showed significant annual changes in the two sexes, except condition factor (K) in male. Excepting the gonado-somatic index, the various indices showed insignificant differences between the sexes. Variations in calorific value of trunk muscle, liver, gut and gonad were found significant over the year. The pattern of changes in the calorific value of muscle, gut and liver were also more or less identical in the two sexes. Calorie deposition in testes remained significantly lower than in the ovaries.

	Condition Factor 'K'		Gonado-somatic Index		Gastro-somatic Index		Liver-somatic Index	
	Male	Female	Male	Female	Male	Female	Male	Female
March, 1988	00.45 ±0.04	00.49 ±0.02	00.10 ±0.02	00.81 ±0.17	01.59 ±0.21	02.04 ±0.80	00.81 ±0.19	00.85 ±0.07
April	00.50 ±0.03	00.50 ±0.04	00.13 ±0.06	00.60 ±0.12	00.94 ±0.13	01.34 ±0.13	00.87 ±0.07	00.93 ±0.09
May	00.51 ±0.06	00.55 ±0.01	00.17 ±0.04	03.81 ±1.42	01.21 ±0.15	01.19 ±0.16	00.94 ±0.08	01.09 ±0.15
June	00.49 ±0.03	00.64 ±0.09	00.53 ±0.11	17.18 ±3.48	01.07 ±0.34	00.98 ±0.15	01.17 ±0.12	01.10 ±0.27
July	00.47 ±0.02	00.60 ±0.02	00.83 ±0.10	23.13 ±0.98	00.78 ±0.22	00.87 ±0.19	01.37 ±0.33	01.13 ±0.19
August	00.42 ±0.03	00.52 ±0.05	00.48 ±0.11	10.86 ±3.94	01.49 ±0.32	01.79 ±0.21	01.42 ±0.29	01.21 ±0.19
September	00.47 ±0.02	00.54 ±0.04	00.15 ±0.04	03.53 ±1.34	01.51 ±0.90	01.81 ±0.20	01.35 ±1.13	01.40 ±0.14
October	00.49 ±0.03	00.51 ±0.05	00.07 ±0.02	00.70 ±0.20	01.49 ±0.23	01.85 ±0.20	01.06 ±0.07	01.21 ±0.22
November	00.48 ±0.05	00.52 ±0.04	00.04 ±0.01	00.59 ±0.07	01.49 ±0.07	02.00 ±0.32	01.09 ±0.15	01.30 ±0.27
December	00.50 ±0.03	00.53 ±0.03	00.03 ±0.00	00.74 ±0.06	01.15 ±0.49	01.62 ±0.14	01.08 ±0.18	01.26 ±0.13
January, 1989	00.49 ±0.03	00.54 ±0.03	00.04 ±0.01	00.65 ±0.11	01.25 ±0.49	01.56 ±0.22	01.07 ±0.33	01.29 ±0.39
February	00.48 ±0.01	00.48 ±0.02	00.04 ±0.02	00.71 ±0.12	01.70 ±0.17	01.68 ±0.14	00.97 ±0.15	01.05 ±0.13

± SEM

Table 2. Seasonal changes in tissue calorific value of *H. fossilis*

	Muscle		Gut		Liver		Gonad	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
March 1988	05.08 ±0.10	05.13 ±0.07	05.42 ±0.19	05.41 ±0.07	05.43 ±0.01	05.32 ±0.05	02.29 ±0.12	04.83 ±0.43
April	05.11 ±0.01	05.16 ±0.02	05.11 ±0.01	05.11 ±0.04	05.62 ±0.09	05.44 ±0.02	02.87 ±0.06	05.11 ±0.07
May	05.13 ±0.04	05.25 ±0.02	04.94 ±0.07	05.05 ±0.02	05.68 ±0.09	05.54 ±0.06	04.21 ±0.04	05.64 ±0.06
June	05.24 ±0.05	05.27 ±0.08	04.95 ±0.15	05.03 ±0.05	05.61 ±0.04	05.47 ±0.08	04.69 ±0.01	05.81 ±0.05
July	05.23 ±0.03	05.21 ±0.01	04.99 ±0.10	05.00 ±0.12	05.57 ±0.04	05.44 ±0.02	04.90 ±0.01	05.92 ±0.12
August	05.06 ±0.03	05.06 ±0.03	05.04 ±0.02	05.10 ±0.10	05.49 ±0.13	05.49 ±0.04	04.81 ±0.09	05.79 ±0.04
September	05.10 ±0.04	05.25 ±0.03	05.28 ±0.17	05.34 ±0.12	05.41 ±0.05	05.52 ±0.08	03.11 ±0.13	05.73 ±0.09
October	05.17 ±0.07	05.29 ±0.05	05.41 ±0.06	05.48 ±0.07	05.33 ±0.08	05.26 ±0.07	02.39 ±0.08	05.58 ±0.09
November	05.54 ±0.05	05.54 ±0.02	05.67 ±0.04	05.68 ±0.00	05.53 ±0.08	05.42 ±0.02	02.22 ±0.04	05.47 ±0.15
December	05.44 ±0.03	05.57 ±0.06	05.45 ±0.05	05.60 ±0.12	05.67 ±0.04	05.86 ±0.00	02.26 ±0.05	05.24 ±0.06
January, 1989	05.39 ±0.06	05.50 ±0.05	05.38 ±0.00	05.59 ±0.00	05.75 ±0.00	05.98 ±0.02	02.17 ±0.06	05.13 ±0.06
February	05.16 ±0.07	05.24 ±0.00	05.77 ±0.19	05.67 ±0.08	05.79 ±0.01	05.89 ±0.12	02.27 ±0.07	04.71 ±0.05

± SEM

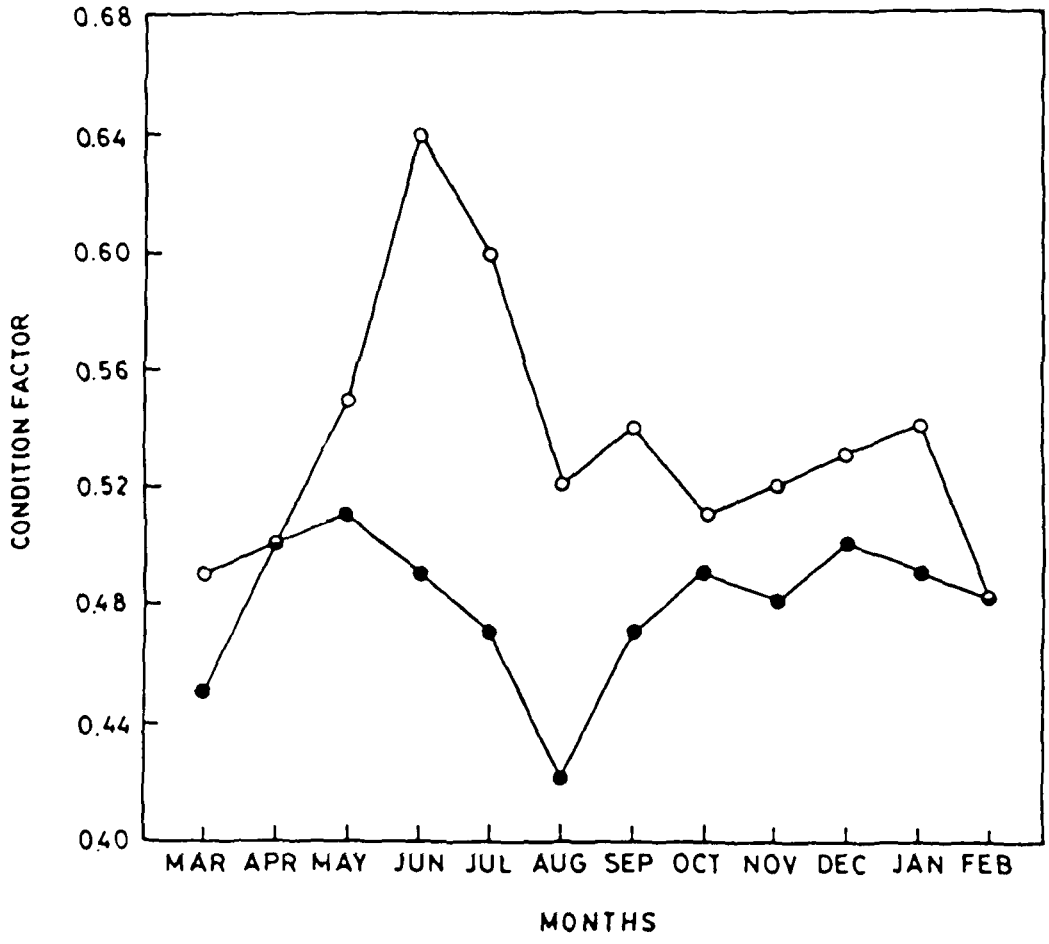

Fig. 1. Seasonal changes in condition factor of mature male (●——●) and female (o——o) H. fossilis.

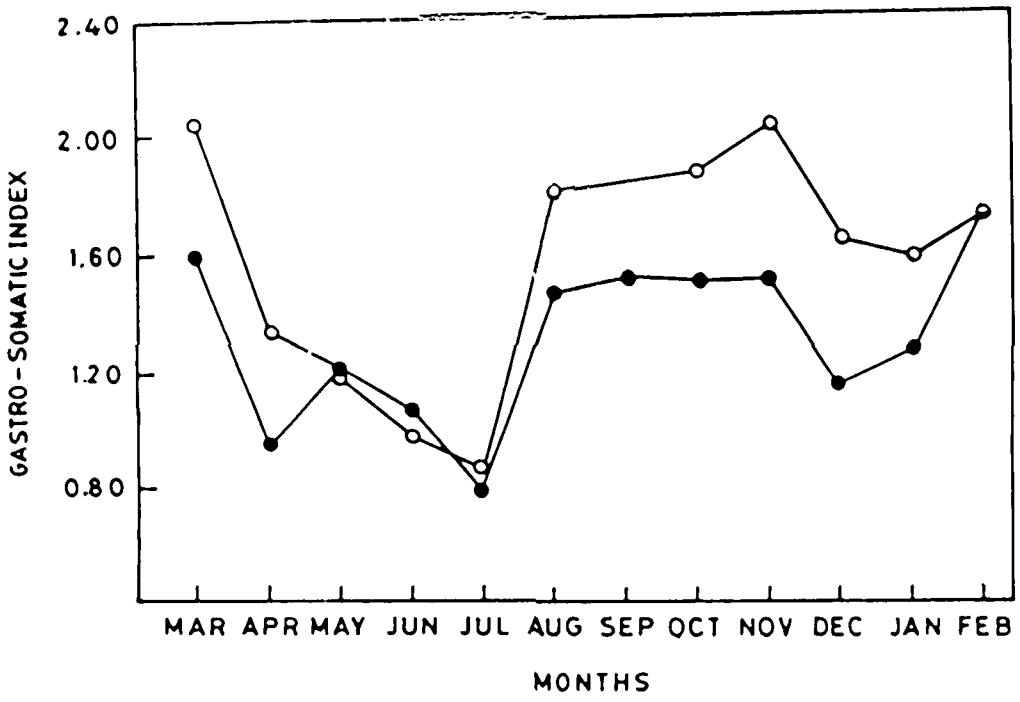

Fig. 2. Seasonal changes in gastro-somatic index of mature male (●——●) and female (o——o) H. fossilis.

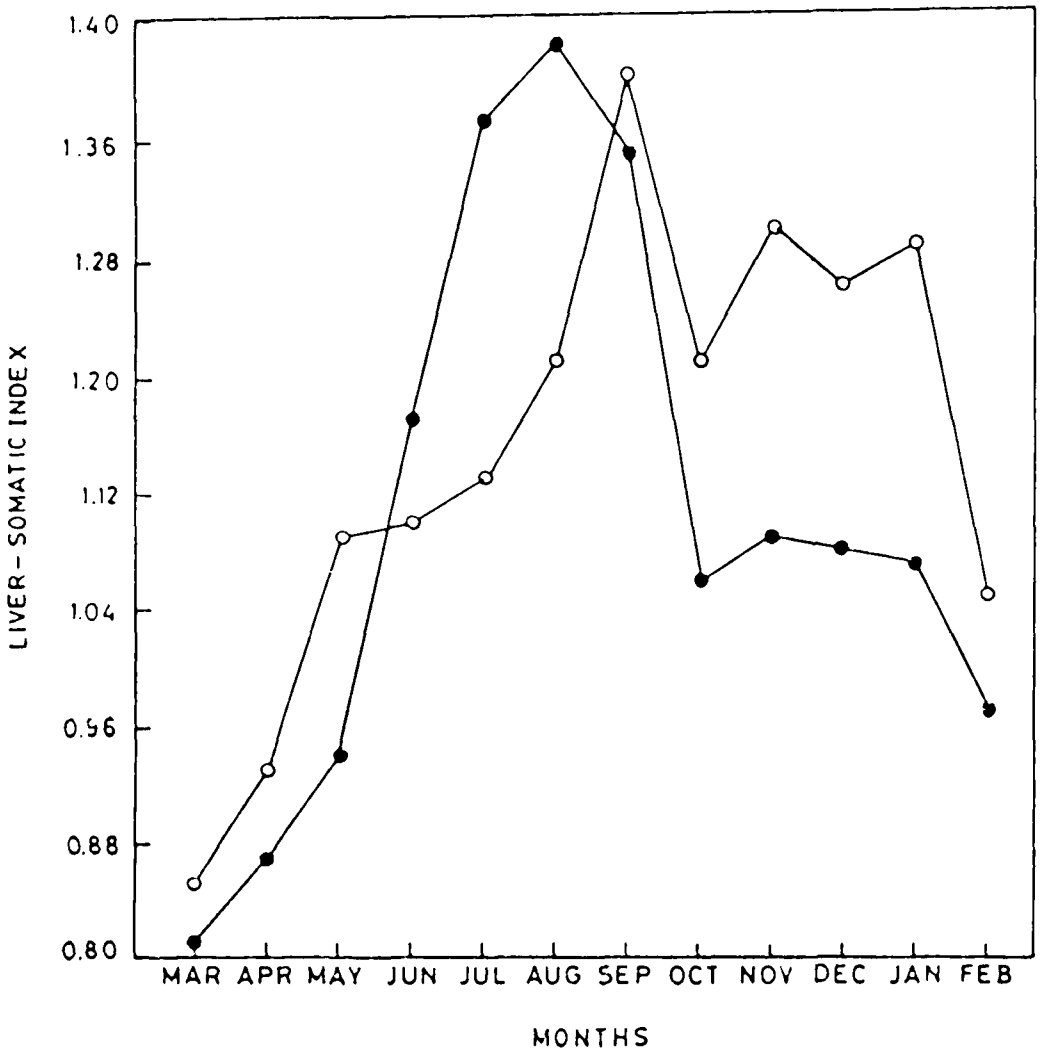

Fig. 3. Seasonal changes in liver-somatic index of mature male (●——●) and female (○——○) H. fossilis.

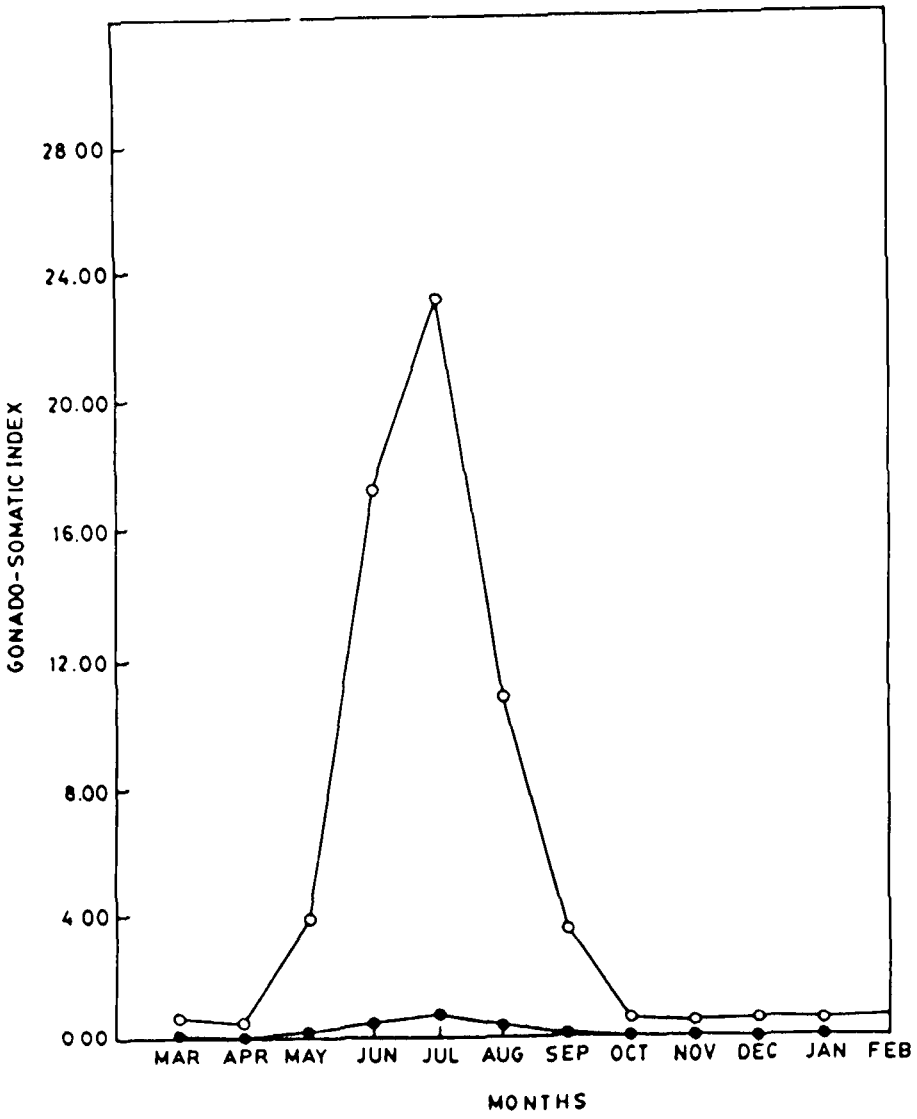

Fig. 4. Seasonal changes in gonado-somatic index of mature male (●——●) and female (○——○) H. fossilis.

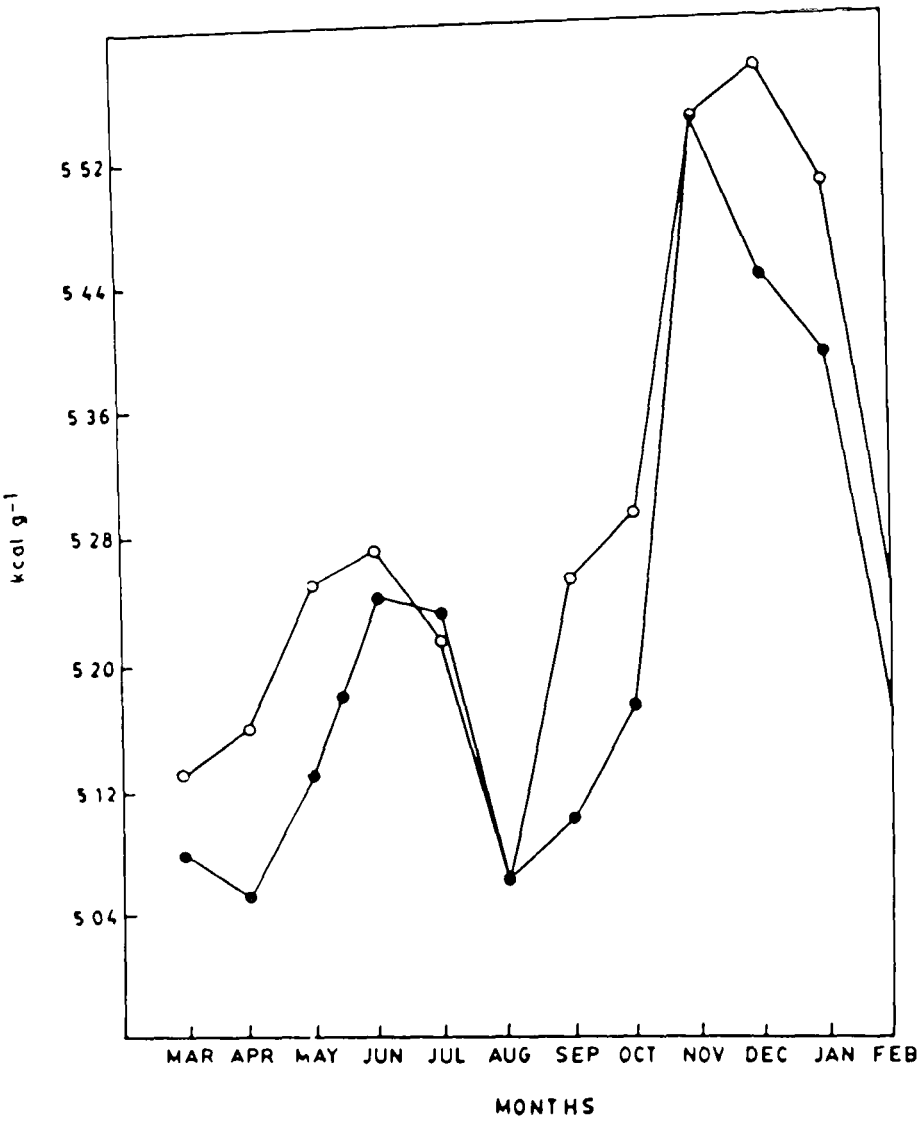

Fig. 5. Seasonal changes in muscle calorific value of mature male (●——●) and female (○——○) H. fossilis.

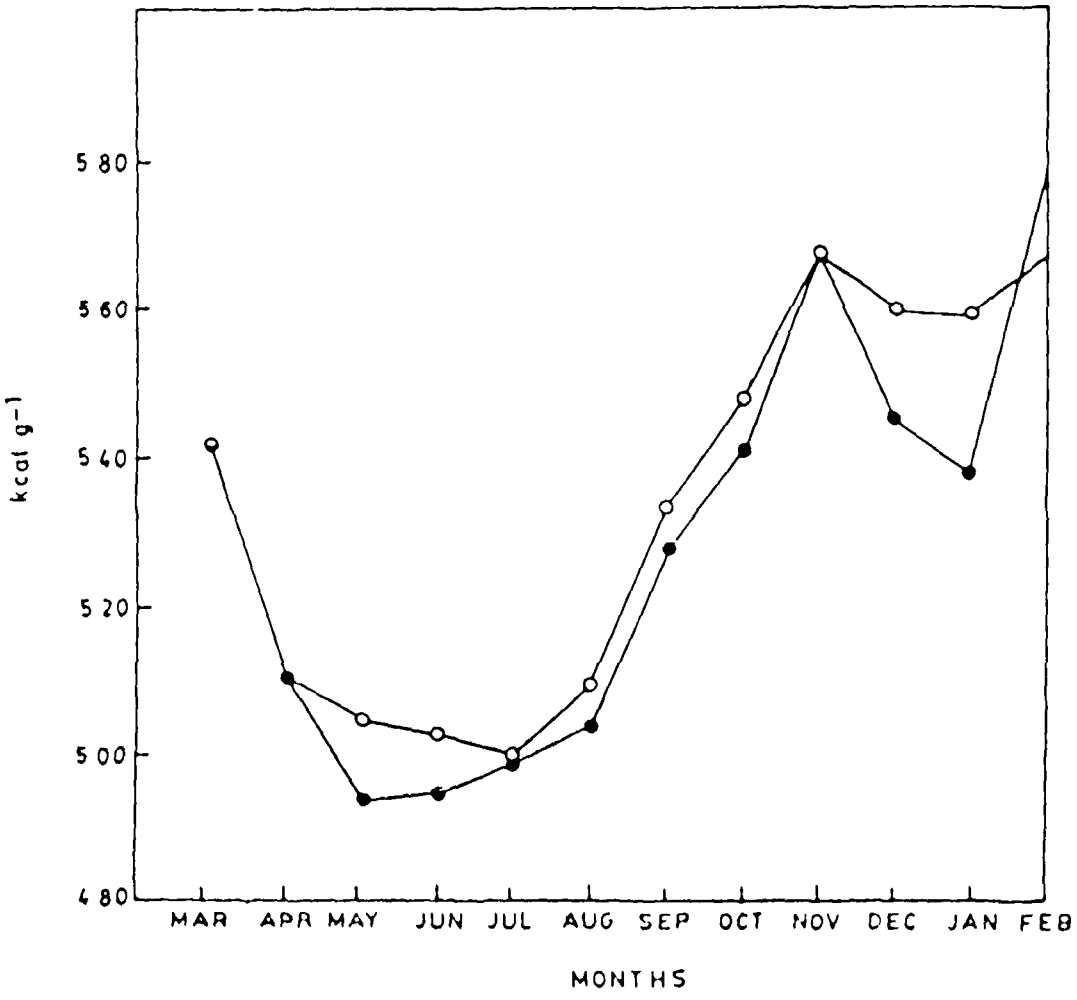

Fig. 6. Seasonal changes in gut calorific value of mature male (●——●) and female (○——○) H. fossilis.

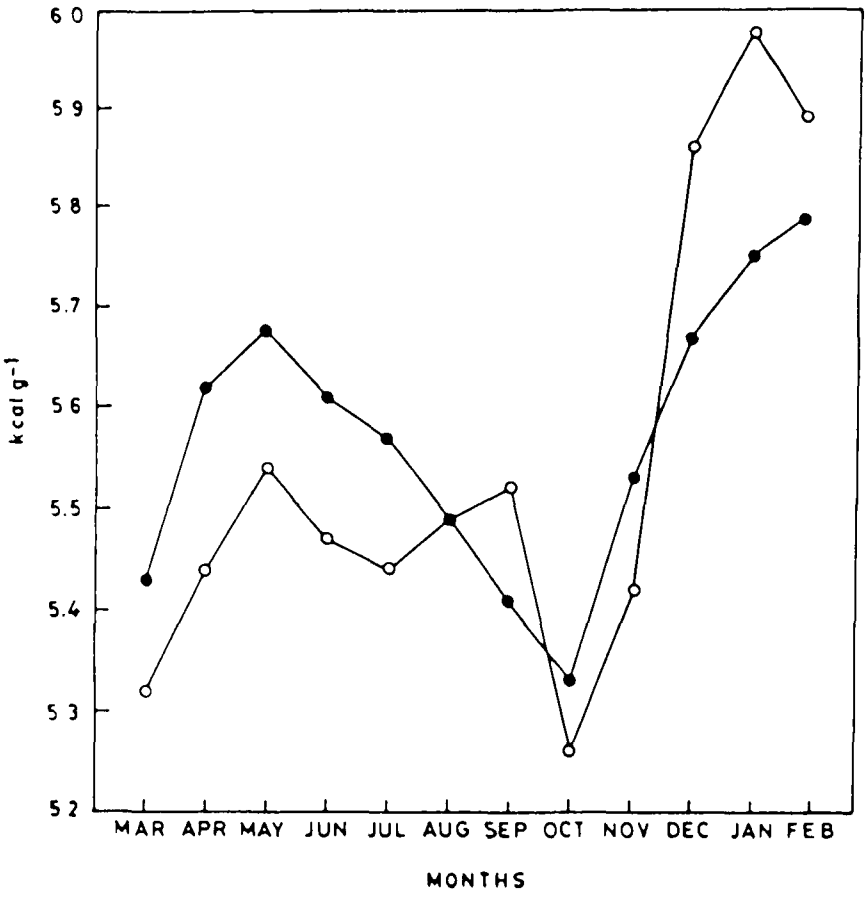

Fig. 7. Seasonal changes in liver calorific value of mature male (●——●) and female (○——○) H. fossilis.

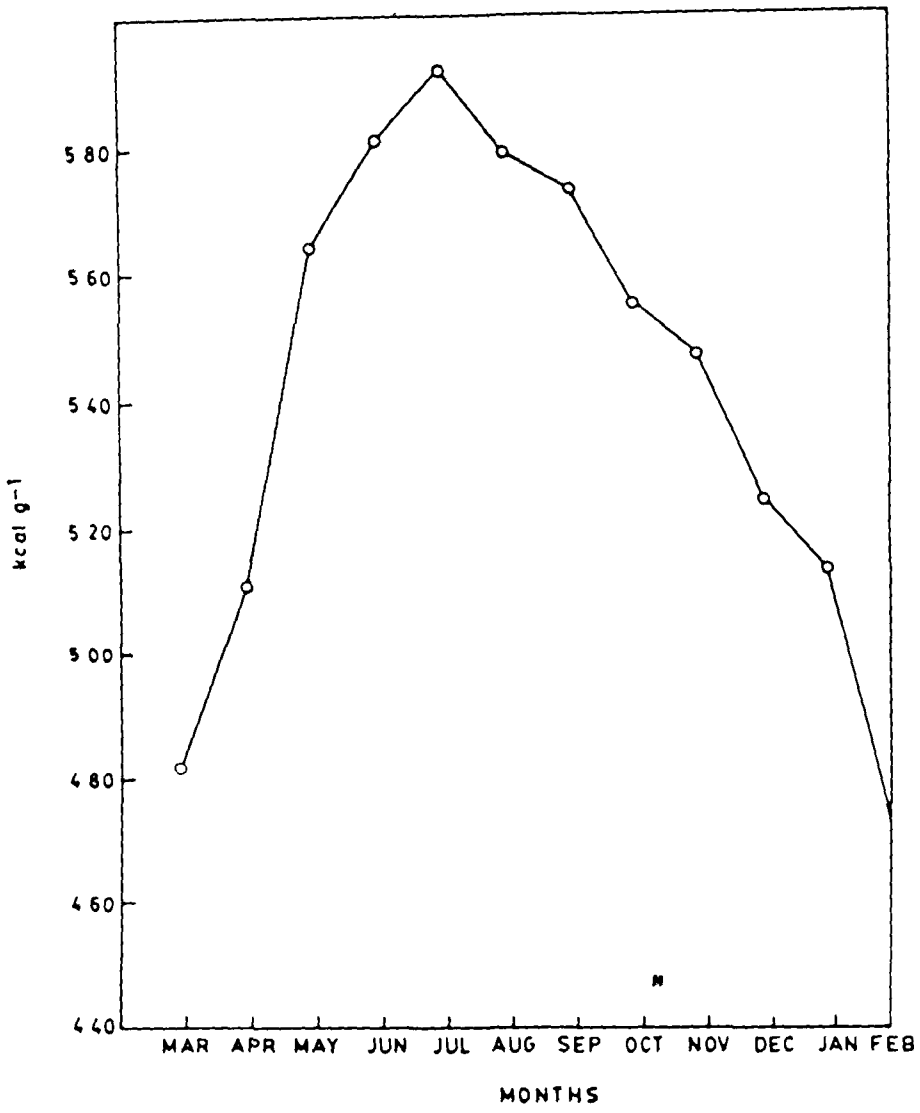

Fig. 8. Seasonal changes in ovarian calorific value of mature female (o——o) H. fossilis.

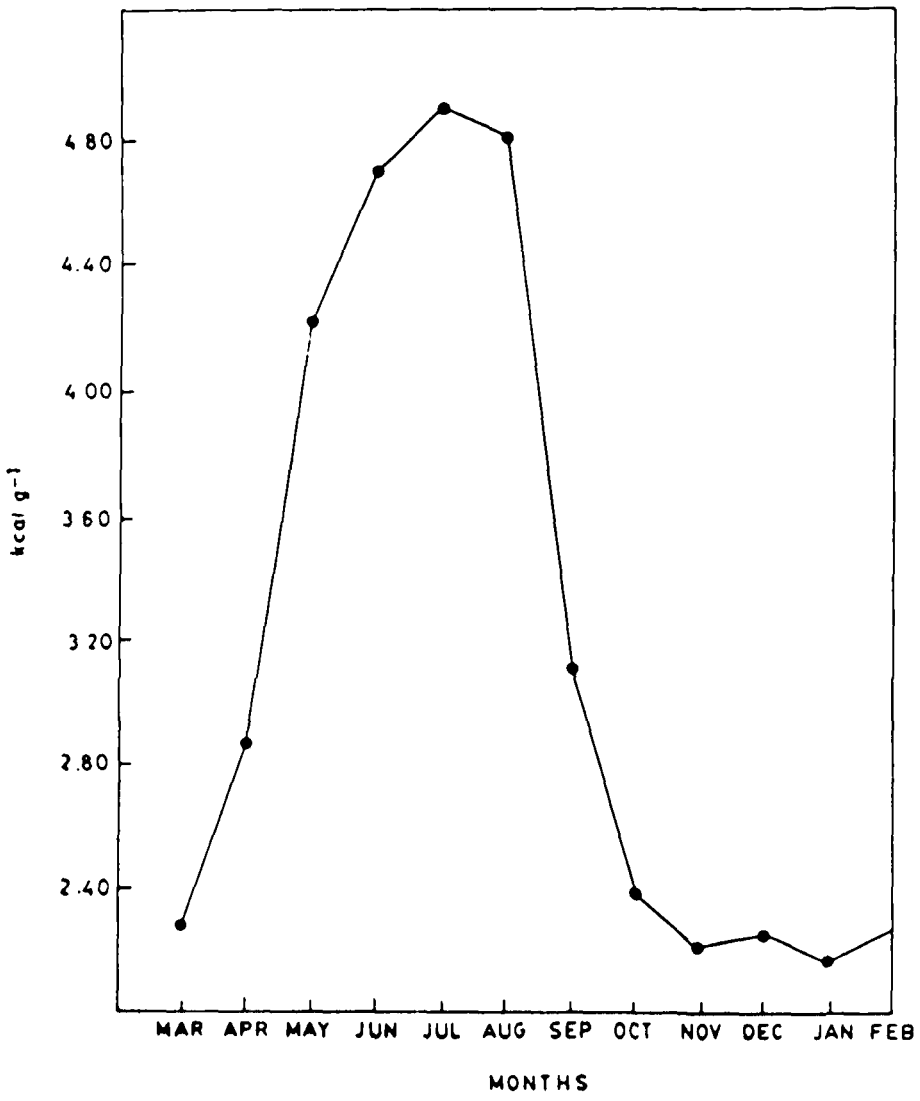

Fig. 9. Seasonal changes in testicular calorific value of mature male (●——●) H. fossilis.

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